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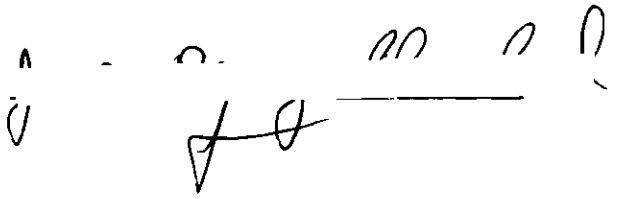

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A STUDY OF SOME ESTERS OF PHOSPHORIC ACID: A RIBONUCLEIC ACID MODEL SYSTEM

A THESIS

Presented to

The Faculty of the Graduate Division

by

James Perry Cleveland

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of the Requirements for the Degree

Doctor of Philosophy

in the School of Chemistry

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April, 1967

A STUDY OF SOME ESTERS OF PHOSPHORIC ACID: A RIBONUCLEIC ACID MODEL SYSTEM

Approved:

Chairman

Date approved by Chairman: 4/20/67

To my patient and loving wife

Jane

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The referencing system used in this thesis is that employed by the American Chemical Society publication Chemical Reviews. The full titles of all journals referred to in the Bibliography can be found in Chemical Abstracts, 50, 1J (1956). Compounds are referred to with underlined Arabic numerals which run consecutively throughout the body of the text. The symbols employed are consistent with those found in the current chemical literature. Since several symbols are regularly referred to throughout various portions of the text, they are listed here for easy reference.

Symbol	Name
12	Ethylene Phosphate
46b	Cyclohexylammonium Methyl cis-3-Hydroxytetrahydrofuranyl-4-phosphate
47b	Cyclohexylammonium cis-3,4-Tetrahydrofurandiol Cyclic Phosphate (Carré's Salt)
48	Barium 2-Bromoethyl Phosphate
49	Barium trans-3-Bromotetrahydrofuranyl-4-phosphate
56	Methyl 2-Hydroxypropyl Phosphate
μ	Micron (Infrared Spectra) or Ionic Strength
J	Coupling Constant (Nuclear Magnetic Resonance Spectra)
IR	Infrared
n.m.r.	Nuclear Magnetic Resonance
k_{2E}	Second-Order Rate Constant for the Base-Catalyzed Hydrolysis of 12

k_2	Second-Order Rate Constant for the Acid-Catalyzed Hydrolysis of Ethylene Phosphoric Acid
k_{2A}	Second-Order Rate Constant for the Base-Catalyzed Racemization of 46b
k_{2B}	Second-Order Rate Constant for the Base-Catalyzed Hydrolysis of 47b
k_a	First-Order Rate Constant for the Cyclization of 48
k_b	First-Order Rate Constant for the Cyclization of 49
$\Delta H^{\neq}$	Activation Enthalpy
$\Delta S^{\neq}$	Activation Entropy
$\Delta G^{\neq}$	Activation Free Energy

SUMMARY

The ribonucleic acids, naturally occurring phosphate diesters, are known to break down by a two-step process. The relatively fast first step is an intramolecular transesterification reaction which gives rise to a series of intermediate nucleotides containing five-membered phosphate diester rings. These intermediate cyclic nucleotides then undergo a relatively slow ring-opening hydrolysis reaction to produce the final nucleotide products. Westheimer and coworkers have shown that the simplest five-membered ring phosphate diester, ethylene phosphate, undergoes hydrolysis in aqueous base by a second-order process at a rate some 10^8 times faster than that of the simplest acyclic analogue, dimethyl phosphate. These workers have further demonstrated that this huge rate difference is associated with "strain" present in the five-membered phosphate ring. This "strain," however, only accounts for a relative rate factor of about 10^4, leaving a rate factor of some 10^4 unexplained. In an effort to delineate the other driving forces for this ring-opening hydrolysis reaction, the activation parameters for the acid- and base-catalyzed hydrolysis of ethylene phosphate have been measured.

An activation enthalpy of 13.1 kcal/mole has been obtained for the base-catalyzed hydrolysis process. The corresponding value obtaining for the dimethyl phosphate hydrolysis is not known but has been estimated to have a minimum value of 19 - 20 kcal/mole. The comparison of these two values then provides a strong argument that the "strain" present in the ethylene phosphate diester ring is larger than the strain value of the

5.5 kcal/mole measured for the methyl ethylene phosphate triester ring. It is suggested that the coulombic repulsions between the two partially negative phosphoryl oxygen atoms produces strain in the cyclic diester relative to the cyclic triester and acyclic diester. Activation free energies of about 23 and 33 kcal/mole have been calculated for the base-catalyzed hydrolyses of ethylene and dimethyl phosphates, respectively. Though the activation entropy for the hydrolysis of the acyclic diester is not known, a consideration of the relative activation free energies and enthalpies suggests, but does not prove, that a favorable activation entropy term is associated with the hydrolysis of the cyclic vis á vis the acyclic diester, assuming, of course, that the two hydrolytic transition states are quite similar electronically. This suggestion is in accord with the postulate that the five-cyclic phosphorus atom bears an enhanced positive charge relative to the acyclic phosphorus atom. An activation enthalpy of 12.5 kcal/mole has been obtained for the acid-catalyzed hydrolysis of ethylene phosphate. Unfortunately, sufficient comparison data for the analogous hydrolysis of the acyclic ester are unavailable.

In order that the hydrolytic reactivity of the intermediate cyclic nucleotides be better understood, the five-cyclic cis-3,4-tetrahydrofurandiol cyclic phosphate has been studied. This ester, which contains the basic skeleton of the ribose sugar ring, hydrolyzes in aqueous base by a mechanism closely akin to that operating in the ethylene phosphate hydrolysis. Furthermore, the tetrahydrofuranyl ester hydrolyzes only 1.8 times faster than does ethylene phosphate while the activation parameters for the two hydrolyses are almost identical! Hence, the tetrahydrofuran

ring has almost no effect upon the reactivity of the five-membered phosphate ring.

It is well known that those esters possessing a β-hydroxyl group undergo transesterification reactions in the presence of aqueous base at enhanced rates relative to the hydrolysis rates of simple, acyclic diesters. In all cases where comparison data are available, however, the rate of the transesterification ring closure is less than the rate of the subsequent ring-opening hydrolysis of the intermediate five-cyclic esters. In order to evaluate the effect of the ribose ring on the transesterification step of the ribonucleic acid breakdown, the rigid methyl cis-3-hydroxytetrahydrofuranyl-4-phosphate has been prepared and studied. In this system the base-catalyzed transesterification ring-closure reaction proceeds at almost the same rate as the hydrolysis ring-opening reaction. Thus, the tetrahydrofuran ring has a very large effect upon the ring-closure reaction! This behavior is much more in line with the ribonucleic acid hydrolysis than the behaviors of other simpler model systems.

It has been assumed that the β-hydroxy esters show enhanced reactions rates because of an entropy advantage resulting from the close proximity of the β-hydroxy group to the reaction center. The activation parameters for the base-catalyzed transesterification ring closure of the β-hydroxy tetrahydrofuranyl ester have, therefore, been measured and compared with the corresponding values published by Brown and coworkers for other β-hydroxy esters as well as those estimated for the base-catalyzed hydrolysis of dimethyl phosphate. These comparisons demonstrate that the rate enhancements observed for the cyclizations of comparable β-hydroxy esters are associated to great measure with activation

enthalpy advantages of about 3.5 - 5.5 kcal/mole. A solvent deuterium isotope effect of 1.06 has been found for the base-catalyzed transesterification of the β-hydroxytetrahydrofuranyl ester, thus implying the intermediacy of alkoxide anions. The enthalpy advantages must then be due primarily to a rather large nucleophilicity difference between the alkoxide and hydroxide anions. Since the activation entropy for the base-catalyzed hydrolysis of dimethyl phosphate is not known, the postulated activation entropy advantage has not been proved.

The activation enthalpy and entropy (13.7 kcal/mole and -27 e.u.) pertaining to the base-catalyzed transesterification of the β-hydroxytetrahydrofuranyl ester have been compared with the activation data (15.7 kcal/mole and -32 e.u.) for the cyclization of the simpler analogue, methyl 2-hydroxypropyl phosphate. The observed activation enthalpy difference probably arises from the relative differences between the ground and transition states of the conformationally rigid and non-rigid esters. The relative enthalpies for the alkoxide ion formation equilibria may also be involved. An activation entropy difference is expected on the basis of the rigid vs. the non-rigid conformations of the two esters. As predicted, the rigid ester shows the more positive activation entropy. Unfortunately, however, this measured activation entropy difference refers to both a rate step and an equilibrium step.

The internal S_N2 displacement reactions of 2-bromoethyl phosphate and trans-3-bromotetrahydrofuranyl-4-phosphate have therefore been studied. The activation entropy obtaining for the S_N2 cyclization of the tetrahydrofuranyl bromohydrin phosphate (+5 e.u.) was more positive than that obtaining for the cyclization of the acyclic bromohydrin phosphate (+2

e.u.). A quantitative extension of this result to the transesterification reactions is, of course, not valid. Nevertheless, the relative activation entropy value for the S_N2 displacement reactions certainly implies that the observed activation entropy difference obtaining for the internal transesterification reactions of the β-hydroxy esters must be at least partially associated with the favorable conformational rigidity imposed by the tetrahydrofuran ring.

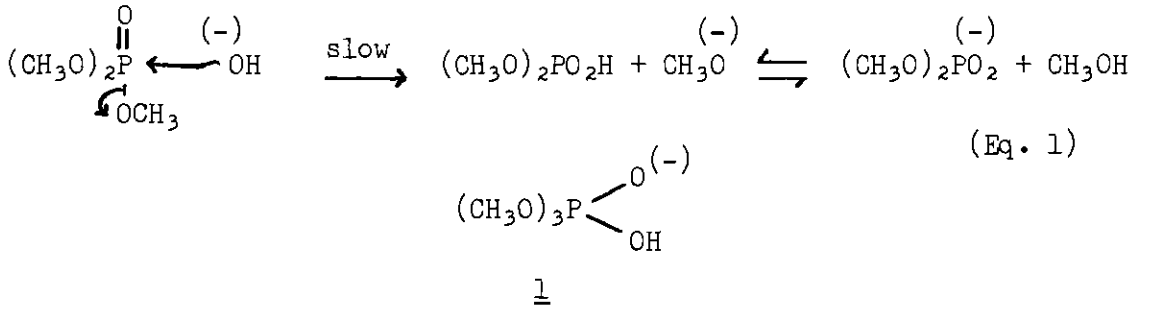
CHAPTER I

INTRODUCTION

Esters of phosphoric acid are known to be vital constituents of living organisms (28,74). Until recently the chemistry of phosphate esters has remained unexplored due largely to such problems as synthetic and purification procedures, multiplicity of their reactions, and a knowledge of physical organic chemistry in general. As these problems have been overcome, a detailed knowledge of the reactions of these compounds has begun to emerge. Indeed, only through an intimate understanding of the mechanistic details of the chemistry of phosphate esters can the chemistry of life processes be elucidated.

Mechanisms of Phosphate Ester Solvolyses (34)

Though there are many data on the solvolyses of many phosphate triesters (34), only the solvolyses of trimethyl and triphenyl phosphates have been studied in detail. Bunton and coworkers (9) have shown that trimethyl phosphate hydrolyzes in aqueous base by a process which is first order in both ester and hydroxide ion. Isotopic labeling experiments have shown that no O^{18} from the solvent is incorporated into the ester prior to the hydrolysis and that the phosphorus-oxygen bond is broken exclusively (16). On changing the solvent from water to 75% dioxane-25% water, a small rate depression is observed. These observations show that the hydrolysis proceeds by the nucleophilic attack of hydroxide ion on phosphorus with the direct expulsion of a methoxide ion (Eq. 1). Though

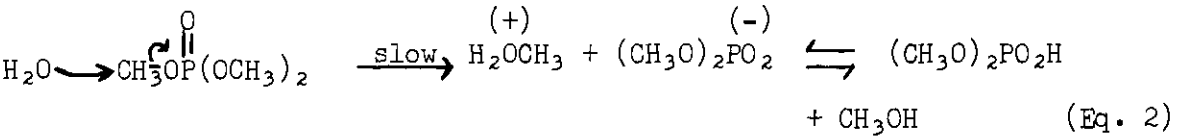


(Eq. 1)

1

pentacoordinate phosphorus compounds are well known (94), a pentacoordinate intermediate, 1, in this system is undetectable experimentally.

Trimethyl phosphate does not appear to be subject to acid catalysis. The results of Bunton and coworkers (10) show that the hydrolysis of this ester in neutral and acidic media proceeds with exclusive carbon-oxygen fission. The mechanism appears, therefore, to be an S_N2 displacement of the dimethyl phosphate ion by water (Eq. 2). As branching on



(Eq. 2)

the alpha carbon increases, a mechanism change is observed. Whereas trimethyl, and presumably, triethyl phosphate solvolyze by an S_N2 reaction at carbon, tri-t-butyl phosphate solvolyzes by an S_N1 mechanism, analogous to the solvolysis of t-butyl chloride (91). The behavior of tri-i-propyl phosphate toward solvolysis is probably similar (91).

A comparison of the specific rate constants for attack of hydroxide ion and water at phosphorus in triphenyl phosphate (75% dioxane - 25% water, 100°) shows hydroxide to be a better nucleophile than water toward

phosphorus by a factor of about 18^8 (10). The corresponding factor toward carbon is only 10^4 (10). Therefore, where possible, hydroxide ion prefers to attack phosphorus.

When an alkoxide anion is the nucleophile attacking a triester, transesterification reactions result with the establishment of equilibria among the various possible products (96). The mechanism of these processes has not been studied in detail although it is probably quite similar to the hydroxide ion-catalyzed hydrolysis mechanism (9,16).

Triesters are usually inert toward attack at phosphorus by nucleophiles other than oxygen anions (34). However, as the basicity of the leaving group decreases, the rate of nucleophilic attack on phosphorus increases. As the basicity of the leaving group increases, the rate of attack on phosphorus decreases, and attack is shifted toward carbon. Miller (88) has studied the relative rates of nucleophilic attack on phosphorus in 0,0-diphenyl phosphorochlorothioate by a series of sulfur and oxygen anion nucleophiles. This remarkable correlation between reaction rate and nucleophile basicity demonstrates that the basicity of a nucleophile is a good measure of its nucleophilicity toward phosphorus. Extraordinary nucleophilicity of anions possessing an atom with unshared electrons adjacent to the nucleophilic center is also observed (88). This behavior, termed the "α effect" (47), has been suggested to arise from the greater electron availability for bond formation although bifunctional catalysis has not been eliminated (88).

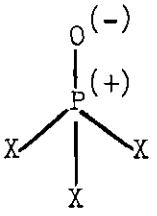
Hudson and Harper have observed that the nucleophilicity of a series of attacking species is proportional to the redox potential of each species (67). Hydroxide ion, however, is about 1000 times more reactive

than predicted. These workers have therefore concluded that the nucleophiles water, chloride, bromide, thiocyanate, iodide, and thiosulfate, attack carbon, whereas hydroxide ion attacks phosphorus. The relative positions of attack of water and hydroxide ion have been confirmed (10).

The available data confirm that the position of nucleophilic attack on a triester is a sensitive function of both steric and electronic factors (34,91). Increased steric crowding can lead to a change of mechanism (9); an alteration in the electronic nature of the leaving group can lead to a change in the point of nucleophilic attack (34). Though it is generally not possible to unravel completely the electronic and steric effects upon phosphate ester reactivity, the results tabulated by Cox and Ramsay (34) taken with the simple electronic bonding picture given by Lucken and Whitehead (84) are of value.

In the Lucken-Whitehead model of a simple trisubstituted phosphorus compound, the molecule is assumed to be constructed with four σ bonds connecting the central sp^3 hybridized phosphorus atom to the four substituents. The z-axis lies along the phosphoryl bond with phosphorus at the origin of the coordinate system. The filled p_x and p_y orbitals on oxygen lie parallel to the xy plane and coincide with the x and y axes of the model, respectively. The empty phosphorus d_{xz} and d_{yz} orbitals then have the correct symmetry for overlap with the occupied oxygen p_x and p_y orbitals. This overlap acts to transfer charge from the negative phosphoryl oxygen atom to the positive phosphorus atom. Charge transfer is far from complete, however, and the oxygen and phosphorus atoms retain a net negative and positive charge, respectively. Of the three remaining phosphorus d orbitals, the d_{xy} and $d_{x^2-y^2}$ orbitals possess the correct symmetry

for overlap with the filled p orbitals of the substituents. The overall effect of the $d_{\pi}-p_{\pi}$ bonding is, therefore, the reduction of the positive charge on phosphorus. In terms of resonance theory, structures 2 and 3 are the most significant contributors to the total structure of the molecule. The three contributing structures designated by 4 are also important but less so than 2 and 3 because the three substituents lie below the xy plane containing the phosphorus atom. Structure 5 makes little or no contribution to the total molecular structure.



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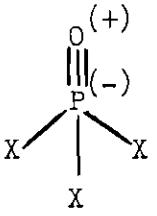

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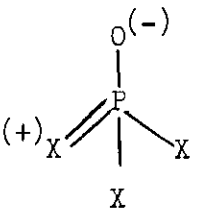

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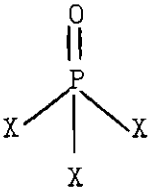

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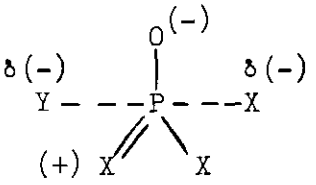

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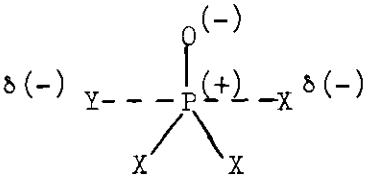

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Although this picture is of a qualitative nature, when coupled with a feeling for the inductive effects of various substituents it greatly aids the understanding of the chemistry of phosphorus compounds. Structures 2 and 3 help to rationalize the large bond strength of the phosphoryl phosphorus-oxygen bond, which has been estimated to be worth about 130-160 kcal/mole (61,89). Although fluorine is more electronegative

than the other halogens, its π bonding ability is much better. Hence, a phosphoryl fluoride is attacked by nucleophiles more slowly than an analogous phosphoryl chloride., Nitrogen is better able than oxygen to form π bonds to the central phosphorus atom and hence reduce its attraction for a nucleophile. This phenomenon, reinforced by the difficulty of separating an amide anion, explains the inertness to alkaline hydrolysis of the phosphoramides relative to the phosphate esters. Similarly, the phosphate esters are more stable to base than are the phosphoryl halides. The replacement of oxygen by sulfur in an ester linkage results in the decreased ability of the esterifying atom to form π bonds with the central phosphorus atom. Mercaptide anions are also better leaving groups than alkoxide anions; hence, the phosphorothiolates are attacked by nucleophiles at phosphorus faster than the analogous phosphates. The substitution of a less electronegative methylene group for the more electronegative oxygen atom in a phosphate eliminates one of the possible d_{π}-p_{π} bonds illustrated by structure 4. The net result of such a substitution is a slight increase in positive charge on phosphorus, and the phosphonates are therefore saponified slightly faster than the analogous phosphates (see reference 34 for a tabulation of experimental data). Substitution of the less electronegative sulfur atom for the more electronegative oxygen atom in the phosphoryl group decreases the positive character of the phosphorus atom through an inductive effect. The phosphorothioates are therefore not attacked by nucleophiles as fast as the corresponding phosphates.

Cox and Ramsay (34) have pointed out that substituents forming weaker d_{π}-p_{π} bonds give rise to a more polar ground state than that

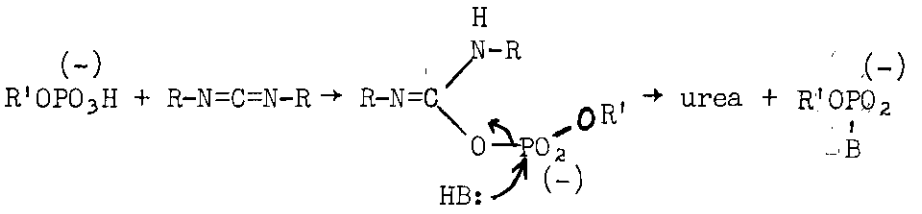
resulting from substituents forming stronger d_{π}-p_{π} bonds (structure 4). Therefore, the more polar is the ground state, the more polar is the transition state required to be (Kekulé formulas 6 and 7). However, the more polar transition state requires a greater amount of solvent ordering than the less polar transition state. Thus, on hydrolysis, esters showing an enthalpy advantage should suffer from an entropy disadvantage, and vice versa. Aksnes has observed these trends in the activation parameters for nucleophilic attack on a series of phosphoryl compounds and has substantiated this interpretation on the basis of enthalpies and entropies of hydrogen bond formation between some of these phosphoryl compounds and phenol (1). Ginjaar and Blasse-vel have also observed these trends in the activation parameters for the alkaline hydrolysis of a series of phosphates, phosphonates, and phosphinates (52).

It is generally observed that an increase in bulk around a phosphorus atom in compounds of the same structural type reduces the rate of nucleophilic attack at phosphorus. This effect is most pronounced when α-hydrogen atoms are replaced by alkyl groups. Thus, dimethyl methylphosphonate hydrolyzes in base at about twice the rate of dimethyl ethylphosphonate (67,68). It must be noted, however, that the different inductive effects of the various alkyl groups also affect the rate of attack at phosphorus. Cox and Ramsay mention other examples of steric effects associated with phosphate ester reactivity (34).

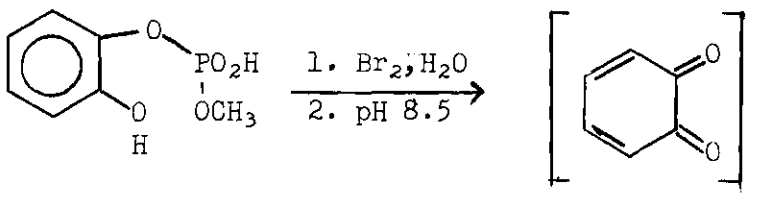
In sharp contrast to the behavior of triesters, the diesters of phosphoric acid (except those either in a five-membered ring or possessing a β-hydroxyl group) are quite resistant to hydrolysis, both acid- and base-catalyzed (75). Thus, Cox (32,77) has observed that the dimethyl

phosphate anion hydrolyzes in base extremely slowly (about 10^{-7} times as fast as trimethyl phosphate in water at 35°) by a process which is first order in ester and in hydroxide ion. Haake and Westheimer (56) have observed about 90 per cent carbon-oxygen cleavage and only about 10 per cent phosphorus-oxygen cleavage in this hydrolysis. These results are in contrast to the 100 per cent phosphorus-oxygen cleavage observed in the base-catalyzed hydrolysis of trimethyl phosphate (16). This low value for the amount of phosphorus-oxygen cleavage is certainly the result, at least partially, of the large coulombic repulsions between the negative nucleophile and the phosphate anion. The monoanion of di-p-nitrophenyl phosphate also reacts very slowly with hydroxide ion in aqueous ethanol (72). The position of cleavage is not known.

If a diester can be "activated," it can be used as a synthetic phosphorylating agent (74). "Activation" may be accomplished, for instance, by incorporating as a leaving group the anion of a dissubstituted urea (Eq. 3), or a quinone (Eq. 4) (73).



(Eq. 3)



(Eq. 4)

Bunton and coworkers (25) have studied the hydrolysis of dimethyl phosphoric acid in solutions varying in acidity from pH 4 to 5M perchloric

acid at 100°. These workers have observed that the hydrolysis of this ester in acid solution, as in basic solution, is quite slow relative to the triester, trimethyl phosphate. In solutions less acidic than pH 0 the only significant reaction is the hydrolysis of the neutral dimethyl phosphoric acid. In solutions 1 to 5M in perchloric acid, however, the hydrolysis of the conjugate acid becomes significant. The observed rate constant at zero ionic strength is given by equation 5, where k_N and k_A have the values 4.92×10^{-6} sec.$^{-1}$ and 1.02×10^{-6} M^{-1}sec.$^{-1}$, respectively.

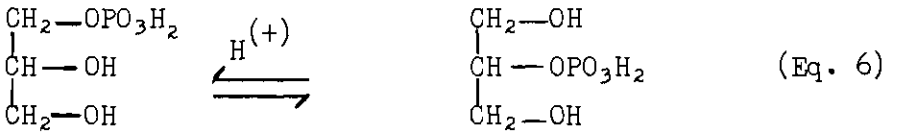
$$k_{obsd} = k_N + k_A[H^{(+)}] \qquad \text{(Eq. 5)}$$

The position of bond fission in the hydrolysis of both the neutral species and its conjugate acid have been investigated by both Bunton's group (25) and Haake and Westheimer (56). The two groups of workers have found that the neutral species hydrolyzes with about 20-30% phosphorus-oxygen cleavage. The corresponding figure for the hydrolysis of conjugate acid appears to be about 0-11%. Kumamoto and Westheimer (78) have observed that the hydrolysis of dibenzyl phosphoric acid, like that of dimethyl phosphoric acid, is also fairly slow and takes place principally through either the neutral species or its conjugate acid.

Cyclic Five-Membered Esters and Their Precursors

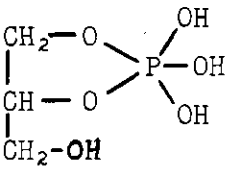
It has long been known that esters possessing a β-hydroxyl group show a greatly enhanced reactivity over esters with no such labilizing group. M. C. Bailly (3) first observed the equilibration of 1- and 2-glycerophosphates in acid solution (Eq. 6). Verkade, Stoppelenburg, and Cohen (104) studied this reaction further and proposed that the interme-

diate cyclic phosphorane, 8, was involved in the equilibrium.



(Eq. 6)

Chargaff (27) showed that the reaction was intramolecular by observing that labeled (P^{32}) inorganic phosphate was not incorporated into the product during the course of the reaction. In more recent work, Kugel and Halmann (76) showed that four oxygen atoms from the solvent were incorporated into the product when the reaction was done in strongly acidic media, thus providing strong evidence that 9 was the detectable intermediate.



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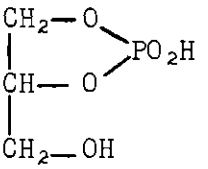


9

O. Bailly and Gaume (5) have observed similar behavior in the base-catalyzed hydrolyses of β-hydroxy di- and triesters. Thus, methyl 2-hydroxyethyl phosphate is rapidly hydrolyzed in base to 2-hydroxyethyl phosphate (Eq. 7); methyl 2-methoxyethyl phosphate, however, is only very slowly hydrolyzed in base to 2-methoxyethyl phosphate (Eq. 8). Furthermore, methyl l-glycerophosphate is also rapidly hydrolyzed in base to a mixture of l- and 2-glycerophosphates (4,6).

This behavior is most striking in the nucleic acid series. Both

$$HOCH_2CH_2O\underset{\underset{OCH_3}{|}}{PO_2}^{(-)} \xrightarrow[\text{fast}]{(-)_{OH}} HOCH_2CH_2OPO_3^{(=)} \qquad \text{(Eq. 7)}$$

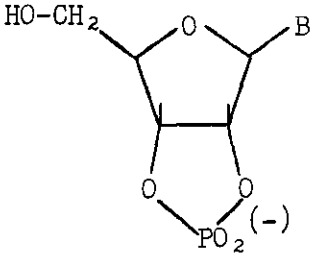
$$CH_3OCH_2CH_2O\underset{\underset{OCH_3}{|}}{PO_2}^{(-)} \xrightarrow[\text{very slow}]{(-)_{OH}} CH_3OCH_2CH_2OPO_3^{(=)} \qquad \text{(Eq. 8)}$$

deoxynucleic acid (DNA) and ribonucleic acid (RNA) are hydrolyzed in acid. DNA is quite resistant to base, while RNA, on the other hand, which contains a β-hydroxyl group, is rapidly depolymerized and subsequently rapidly hydrolyzed under basic conditions to a mixture of the 2'- and 3'-nucleotides (28,85).

Fono (49) and later Brown and Todd (21) were the first to realize the mechanistic significance of these observations. These workers proposed that the depolymerization of the RNA molecule led first to intermediate cyclic nucleotides which were subsequently hydrolyzed rapidly to the observed products. This proposal was also in line with the observations of O. Bailly and Gaume (4,5,6) on the behavior of other β-hydroxy esters in alkaline solution. Markham and Smith, while studying the hydrolysis of the ribonucleic acids with both the enzyme ribonuclease and mild bases, succeeded in identifying intermediate cyclic nucleotides among the early reaction products (85). Todd and coworkers (21) were then able to synthesize these intermediates and to show that they were hydrolyzed in base rapidly enough to fit the proposed mechanistic scheme.

Lipkin, Talbert, and Conn (82) hydrolyzed a ribonucleic acid in O^{18} water and found that only one O^{18} atom from the solvent was incorporated into the final nucleotide products. This result proved that the

intermediate must be a cyclic diester, 10, not a triester as originally proposed (21,49). Furthermore, the result indicated that exclusive



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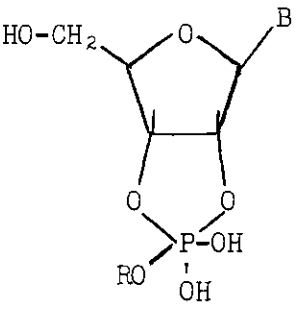
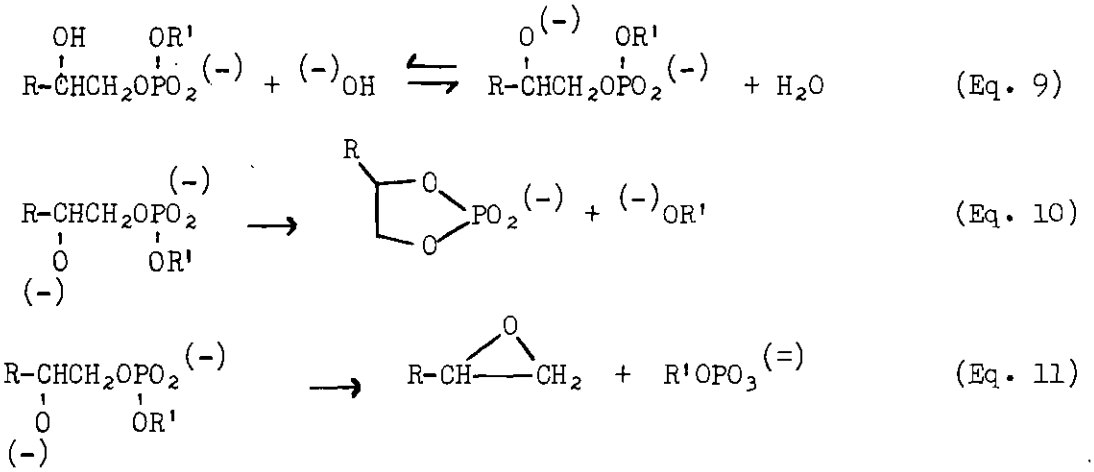


11

phosphorus-oxygen cleavage must have occurred. Todd's group (19) subsequently looked for phosphoryl migration from the 2'- to the 3'-position (or vice versa) during both the acid- and base-catalyzed hydrolyses of the ribonucleic acids. By stopping the reaction at about one half completion, they found that no phosphoryl migration had taken place in the base-catalyzed process. In acid, however, a considerable amount of migration had occurred. These observations indicated that in basic solution a one-step transesterification led from the nucleic acids to the intermediate cyclic nucleotides. The formation of an intermediate cyclic phosphorane (11) in acid was neither eliminated nor confirmed by this result. By analogy with the results of Kugel and Halmann (76), from the study of the O^{18} exchange during the isomerization of the glycerophosphates in acid, a direct displacement of either alcohol or water would appear to be correct. The possible transient existence of intermediate cyclic phosphoranes, however, is still very much open to question (see discussion of five-membered rings below).

Figure 1 represents the currently accepted pathway for the base-catalyzed depolymerization and hydrolysis of a ribonucleic acid.

More recently Brown and coworkers have studied a large number of models for the first step in the ribonucleic acid hydrolysis (17,18,22,33). In these models, where comparison data are available (34,77), the ring-closure step is slow. These results are in direct opposition to the results with the naturally occurring substrates where the ring-closure step is fast. Furthermore, in many of Brown's models extensive epoxidation occurs. These models do behave like the nucleic acids, however, in that they all react at rates significantly greater than the rate of alkaline hydrolysis of dimethyl phosphate. Equations 9, 10, and 11 are illustrative of the reactions studied by Brown's group.

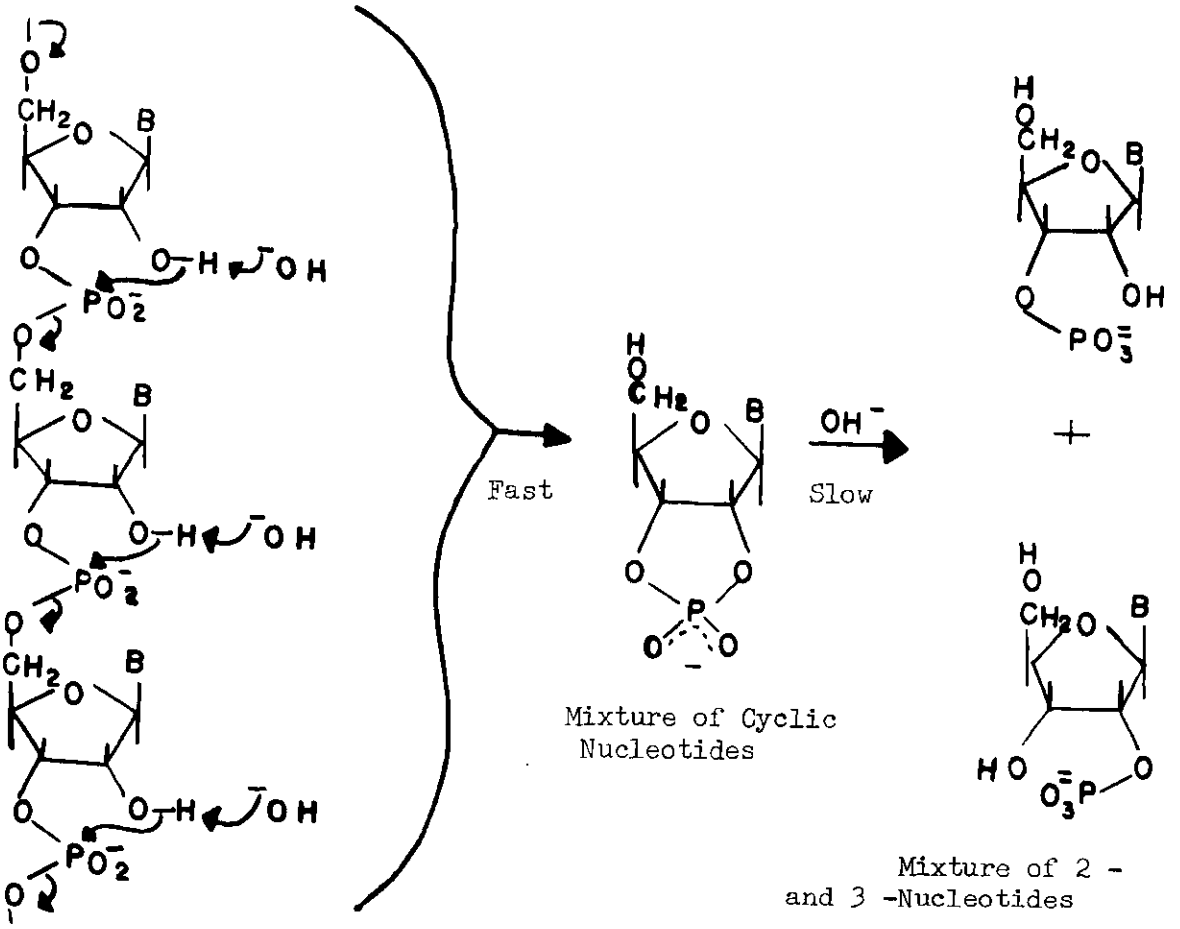


(Eq. 9)

(Eq. 10)

(Eq. 11)

That the β-hydroxy esters show enhanced reactivities over "normal" esters (i.e., those with no labilizing β-hydroxyl group) is due, presumably, to a favorable entropy advantage built into the molecule in the form of this labilizing group. Normal esters, of course, have no such entropy



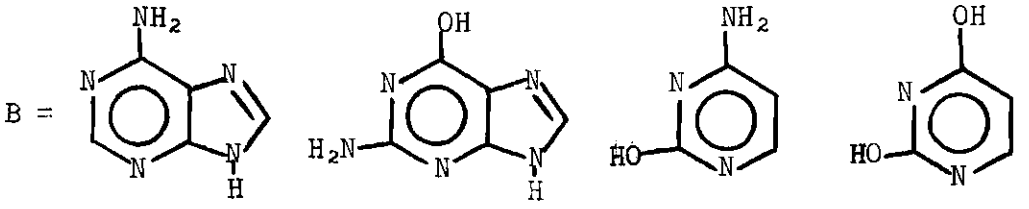


Figure 1. The Base-Catalyzed Depolymerization and Hydrolysis of a Ribonucleic Acid.

"boost." The naturally occurring substrates, in which the β-hydroxyl group is held in a rigid conformation, would be expected to show an even more favorable entropy advantage than the acyclic esters studied by Brown's group. (This aspect of the problem will be dealt with in detail later in this thesis.)

In addition to the activation entropy advantage resulting from the β-hydroxyl function, there may be an activation enthalpy advantage also resulting from this group. The active nucleophiles in the transesterification reactions, the alkoxide anions, are undoubtedly present in fairly small concentration. Nevertheless, they are probably better nucleophiles than hydroxide ion itself toward phosphorus (88) and, once formed (assuming a rapid acid-base equilibrium given by equation 9), should attack the phosphate function faster than the hydroxide ion.

The acid-catalyzed cyclization processes have apparently not been studied. Neither has a general acid nor a general base study appeared. Since ribonuclease may function by a mechanism akin to both general acid and general (or nucleophilic*) base catalyses, these studies are of prime importance.

Since the β-hydroxyl group is primarily responsible for the enhanced rates of the transesterification reactions, one is led to predict that other nucleophilic groups in the β position should function in a qualitatively similar manner. Thus, the many possible reactions illustrated by equation 12 merit investigation.

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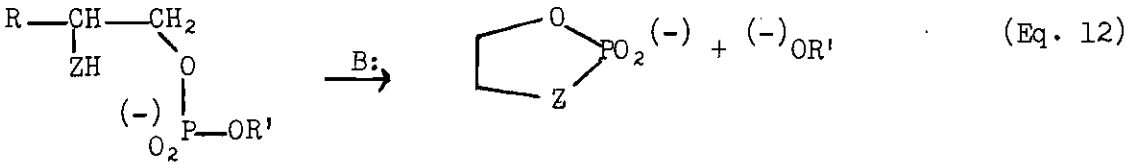
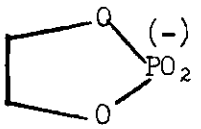
* Westheimer and coworkers have presented evidence that the histidine moiety in some enzymes may act as a nucleophilic catalyst in some phosphate ester solvolyses (15,43).



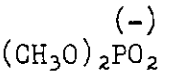
(Eq. 12)

-ZH is a nucleophilic group as a substituted amino or thiol function.

When the extremely reactive, cyclic five-membered phosphate diesters were shown to be intermediates in the ribonucleic acid hydrolysis (82), the source of their high reactivity immediately became a point of investigation. Kumamoto, Cox, and Westheimer (77) succeeded in synthesizing the simplest cyclic five-membered diester, ethylene phosphate, 12, and found that at 25° it was hydrolyzed in base by a second-order process at a rate some 10^7 times faster than the simplest acyclic analogue, dimethyl phosphate, 13. Labeling experiments using an O^{18} solvent showed that the hydrolysis of the cyclic ester proceeded with exclusive phosphorus-oxygen cleavage (56). Thus, attack at phosphorus proceeded at about 10^8 times faster in the cyclic than in the acyclic ester. Furthermore, the rates of alkaline hydrolysis of both ethylene phosphate and the intermediate nucleotides were of a comparable magnitude (77). The extreme lability of the intermediate nucleotides must, therefore, be associated directly with the presence of the five-membered ring.



12



13

The same extremely rapid hydrolysis rates found in the alkaline

hydrolysis of ethylene phosphate were also found in the acid-catalyzed hydrolysis (32,34). In addition, only phosphorus-oxygen cleavage was observed (56). The biggest surprise, however, came from the kinetic order determination. Cox found that the rate law contained only the term for the hydrolysis of the conjugate acid of ethylene phosphoric acid only (Eq. 13), regardless of the acidity of the medium.

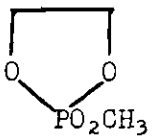
$$V = k_2 [\text{ethylene phosphoric acid}][H^{(+)}] \qquad \text{(Eq. 13)}$$

It should be recalled that the rate law for the hydrolysis of dimethyl phosphoric acid in acid solution contained terms for both the neutral and conjugate acid species (25). Since the two kinetic studies were done at different temperatures, and since the amount of phosphorus-oxygen cleavage in the hydrolysis of the dimethyl phosphoric conjugate acid was not determined precisely, the relative rate factor in acid between ethylene and dimethyl phosphoric acids could not be determined. Visual inspection of the data, however, indicated that the rate factor was roughly of the same order of magnitude in both acid and base.

Haake (56) has made the very important discovery that the acid-catalyzed hydrolysis of ethylene phosphoric acid is accompanied by O^{18} incorporation into the phosphoryl group from the solvent <u>prior to</u> ring opening. Furthermore, the rate of ring opening has been found to be only about five times faster than the rate of O^{18} exchange. The determination of the amount of O^{18} exchange in the hydrolysis of dimethyl phosphoric acid in acid solution is less precise, but the available data (56) indicate that exchange is very slow and may take place through either the neutral species or its conjugate acid. Thus, the inclusion of the ester

in a five-membered ring accelerates the rates of both ring opening and exchange! The driving forces for the hydrolysis and the exchange, therefore, must be approximately equal and be satisfied in the <u>transition states</u> (or <u>intermediates</u>) for the two reactions, even though the final product of exchange is the ring-retained ester. The same conclusion is probably valid for the base-catalyzed hydrolysis even though prior exchange is not observed (56).

The huge reactivity of the cyclic ester was thought to be due to the relief in the transition state of "thermodynamic strain" present in the five-membered ring (35). That this concept was only partially correct was demonstrated by the work of Westheimer's group (35,71) by a comparison of the heat of hydrolysis of the cyclic methyl ethylene phosphate with that of the acyclic dimethyl 2-hydroxyethyl phosphate* (Eq. 14 and 15).



$$\xrightarrow{(-)\ OH} HOCH_2CH_2O\underset{\displaystyle OCH_3}{\overset{\displaystyle (-)}{P}}O_2 \qquad \text{(Eq. 14)}$$

$$HOCH_2CH_2OPO(OCH_3) \xrightarrow{(-)_{OH}} HOCH_2CH_2O\underset{\displaystyle OCH_3}{\overset{\displaystyle (-)}{P}}O_2 \qquad \text{(Eq. 15)}$$

The hydrolysis of the five-membered ring ester was found to be accompanied by the release of about 5.5 kcal/mole of heat in excess to that of the acyclic ester. This "strain" energy, though quite significant, only accounted for a relative rate factor of $\approx 10^4$.

---

* Wall (105) found a huge reactivity difference between the cyclic and acyclic triester roughly comparable to that found in the diester series.

In order to account for the relative rate factor of $\approx 10^4$ not explained by strain energy at least one (and perhaps several) other rate-influencing phenomenon must be operating in the five-membered ring compounds. A more positive charge on the phosphorus atom in the five-cyclic versus the acyclic esters would be manifest in a greater attraction for nucleophiles in the five-cyclic versus the acyclic series. A greater attraction for nucleophiles could then be responsible to a large degree for the large observed rate differences. There is evidence that the phosphorus atom in a five-membered ring is more positive than one in an acyclic or six-membered ester. The P^{31} n.m.r. spectra of several phosphate (14, 70) and phosphonate (46) esters have been recorded. In all cases reported the phosphorus resonance signals of the five-cyclic esters come at a decidedly lower field strength than those of the acyclic series. That the phosphorus atoms in the five-cyclic esters are less shielded than those in the acyclic compounds strongly suggests that the five-membered ring phosphorus atoms bear a more positive charge than do those in the acyclic analogues.

Both increased positive charge on the phosphorus atom in the five-cyclic esters and ring strain are expected from Westheimer's proposal that phosphorus-oxygen d_π-p_π bonding is diminished by constraining the phosphate ester grouping to a five-membered ring (46,71,102). On the basis of the Lucken-Whitehead model (84) the positive charge on a central phosphorus atom in a tetravalent phosphorus compound is a sensitive function of the ability of the substituent groups X to form d_π-p_π bonds with the phosphorus atom (4). If the five-membered ring is then responsible for a diminution of phosphorus-oxygen d_π-p_π bonding, one expects a smaller

contribution from structures represented by 4 to the total structure of the molecule. Thus a more positive charge on the phosphorus atom and a destabilization of the ring would be expected. In terms of molecular orbital theory, the effect of the five-membered ring is that of lessening the overlap integral(s) for the phosphorus-oxygen π bonds.

Evidence for this proposal has come from X-ray crystallography studies. Reasoning from symmetry arguments and the structural parameters for methyl ethylene (98) and methyl pinacol phosphate (92) Newton, Cox, and Bertrand have argued that the structures of the cyclic esters are consistent with the formation of only four π bonds as opposed to the proposed five π bonds in the Lucken-Whitehead model of the acyclic esters. This leaves unoccupied one of the phosphorus d orbitals previously available for π bonding. The presence of one such unoccupied d orbital on phosphorus should then facilitate both nucleophilic attack at phosphorus and the formation of a transition state with sp^3d hydridization. (See the discussion of transition states below.) In this picture, then, the unoccupied phosphorus d orbital provides a low energy pathway for hydrolysis and accounts for the unexplained enhanced reactivity of the five-cyclic esters.

Collin (30) has made some molecular orbital calculations on phosphate esters with regard to d_π-p_π bonding. He has come to the conclusion that π bonding is not only important but is also a sensitive function of the conformation of the ester. Using the known structural parameters of phosphate esters in a self-consistent molecular orbital calculation, and without imposing a priori any symmetry restrictions on the molecules, he has calculated the charge distributions in both five-cyclic and acyclic

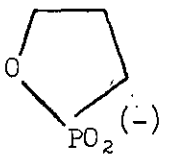
esters. The results of these calculations suggest that the net partial positive charge on phosphorus is larger in the five-cyclic than in the acyclic esters. (See reference 91, page 134.) Collin therefore feels that the increased charge on phosphorus in the five-cyclic compounds is primarily responsible for their large, unexplained reactivity. Though this explanation differs slightly from that given by Newton, Cox, and Bertrand, all four agree that π bonding effects are quite important.

Usher, Dennis, and Westheimer (102), attempting to locate the source of the strain in the five-cyclic esters, have performed some calculations on an assumed planar model, considering only angle and eclipsing strain. These calculations have revealed that from 3-6 kcal/mole of strain energy may be expected in the five-cyclic esters. This is in quite good agreement with the experimentally determined 5.5 kcal/mole observed with methyl ethylene phosphate (35,71). It must be noted that the calculated strain value neither supports nor contradicts the π bonding concept. It is perhaps significant that the differences in observed vs. calculated strain energies (0-2.5 kcal/mole) are not so large as to necessarily preclude an energy term arising from a diminution in π bonding.

If the presence of the ring does diminish any π bonding stabilization, it does not appear internally in each cyclic ester in the form of different esterifying phosphorus-oxygen bond lengths, since these distances in methyl ethylene and in methyl pinacol phosphates are all internally identical (92,98). These observations, of course, may be interpreted as evidence against π bonding effects, or, better, as evidence supporting the view that π bonding effects are diminished in all three

phosphorus-oxygen ester bonds in the five-cyclic compounds (the combined arguments of Collin and Newton, Cox, and Bertrand). In order to make a firm decision from X-ray data about the magnitude of π bonding effects (if any), data on a good model are required. Unfortunately, neither dibenzyl phosphoric acid (45) nor triphenyl phosphate (38) are good models for comparison. Steric effects may be important. Since two of the esterifying groups in the five-cyclic esters are tied back, it can be argued that these esters present less steric hindrance to the approach of an incoming nucleophile than do the acyclic or six-cyclic esters. This effect may be worthy of consideration though it certainly would not appear to be sufficient to explain the observed enhanced reactivities *in toto*. Of course, a steric effect does not account for the apparently increased positive charge on the five-cyclic phosphorus atom.

The enhanced reactivities of the five-cyclic compounds are not restricted to the phosphate series only. Eberhard and Westheimer (46) have observed that the five-cyclic propyl phostonate, 14, hydrolyzes at an enhanced rate relative to the six-cyclic butyl phostonate, 15, and the acyclic ethyl ethylphosphonate, 16. In aqueous base all three esters hydrolyze by a second-order process. Exclusive phosphorus-oxygen cleavage



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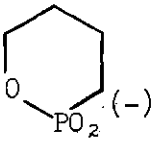

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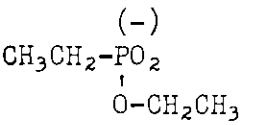
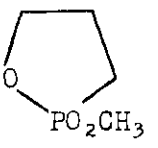


16



17

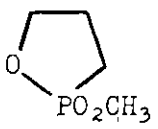
occurs in the two cyclic esters (14) and (15) whereas about 50 per cent

carbon-oxygen cleavage occurs in the acyclic ester (16). In addition, the five-cyclic ester hydrolyzes by attack at phosphorus at a rate some 10^6 times greater than the six-cyclic or acyclic esters.

In aqueous acid the two cyclic esters also hydrolyze with only phosphorus-oxygen cleavage; approximately 60-70 per cent phosphorus-oxygen cleavage obtains for the acyclic 16. The observed rate factor in acid for 14 relative to 15 and 16 is about 10^5. Furthermore, the observed pseudo-first order rate constants for all the acid hydrolyses can be represented by equation 16. Thus, in acid, all three esters hydrolyze through both the neutral and conjugate acid species, a behavior similar

$$K_{obsd} = K_w + K_H [H^{(+)}] \qquad \text{(Eq. 16)}$$

to that observed with the acyclic dimethyl phosphoric acid (25). It should be recalled that ethylene phosphate hydrolyzes in acid only through its conjugate acid. Westheimer has also reported that the fully esterified five-cyclic phostonate, 17, hydrolyzes (conditions not given) some 10^6 times faster than its acyclic analogue and with exclusive ring opening (Eq. 17) (40,46).



17

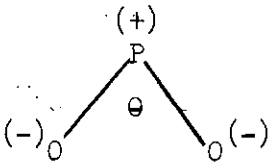
$$\xrightarrow{[H_2O]} \quad HO\text{-}CH_2CH_2CH_2\overset{OCH_3}{\overset{|}{P}}O_2H \qquad \text{(Eq. 17)}$$

The behavior of the phosphonate systems appears to be in accord with the concepts of π bonding and ring strain as applied to the phosphate systems. The n.m.r. spectral data (chemical shifts), taken with

the positions of bond fission observed for the hydrolyses, suggest that the phosphorus atom in a five-membered phosphonate bears a more positive charge than one in either a six-membered or an acyclic phosphonate. (Dipole moments of the five-cyclic vs. the acyclic compounds may be informative in this regard.) This tentative interpretation of these observations may be used as evidence that π bonding is diminished in the five-membered phosphonates relative to the six-cyclic and acyclic analogues. However, any such π bonding diminution is by no means necessarily so large as that proposed for the corresponding phosphate system. Though the five-cyclic phosphonates are probably strained (102), a lack of structural information, theoretical calculations, and thermochemical measurements prohibits a more detailed analysis of the meaning of the kinetic measurements. It is interesting that rate enhancements have also been observed in the hydrolysis of five-cyclic sulfate esters (71).

As previously mentioned, the cyclic triester, methyl ethylene phosphate, shows a huge solvolytic reactivity over its acyclic analogue, trimethyl phosphate. The reported rate factor in the triester series for attack at phosphorus, however, is in the neighborhood of 10^6 (46), not 10^8 as observed for phosphorus-oxygen cleavage in the diester series (77). A factor of only about 100 is therefore not accounted for in the triester series by ring strain. It has been tacitly assumed that the strain observed in the cyclic triester is a good measure of that found in the cyclic diester. This is not necessarily a good assumption. The coulombic repulsion between the two partially negative phosphoryl oxygen atoms may destabilize the cyclic diester ring and appear as strain relative to both the five-cyclic triester and the acyclic diester. The

magnitude of such a coulombic repulsion would, of course, be a function of the phosphoryl O-P-O bond angle θ, in the cyclic vs. the acyclic series. Furthermore, the repulsion should be larger in the diester series (18) than in the triester series (19). There are no structural



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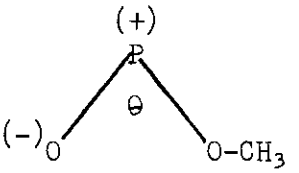
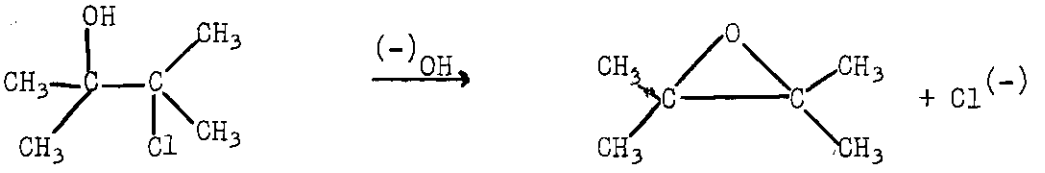
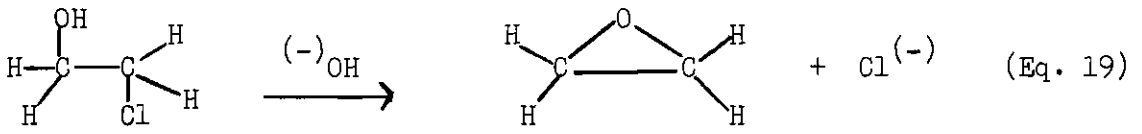


19

comparison data available for appropriate cyclic and acyclic diesters, but the structural parameters for the cyclic methyl ethylene (98) and methyl pinacol phosphates (92), compared with those for triphenyl phosphate (38) and dibenzyl phosphoric acid (45) indicate that such a coulombic repulsion may be significant, particularly in the diester series. The (at least partial) relief of such a coulombic repulsion in the transition state would not appear to be too dissimilar qualitatively to the relief of one or more gem-dimethyl steric repulsions. For example, the relief of two such repulsions in the transition state is thought to be responsible for the huge epoxidation rate of tetramethylethylene chlorohydrin over that of ethylene chlorohydrin (93,99) (Eqs. 18 and 19).



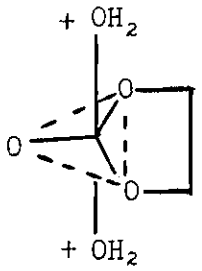
(Eq. 18)



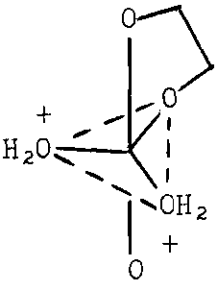
(Eq. 19)

Haake's discovery that the driving forces in the ethylene phosphate ring for both the acid-catalyzed ring opening and O^{18} exchange from the solvent are very nearly the same and are satisfied in the transition states (or intermediates) for the two processes leads to the logical assumption that the mechanisms of both reactions are very nearly the same (56). If the mechanisms of the two reactions are nearly the same, then, of course, the geometries of the two transition states (or intermediates) should be quite similar. Proceeding on this last assumption, the number of possible structures for the two transition states (or intermediates) is quite limited. Formulas 20, 21, and 22 represent three reasonable geometries of the activated complexes (or intermediates) for either the ring opening or exchange reaction. Each formula contains one ethylene phosphoric acid, one extra proton, and one water molecule, in accord with the kinetic equation (32,34). The single positive charge is divided between the oxygen atoms marked +. Structures 20a, 21a, and 22a represent transition states (or intermediates) for O^{18} exchange, 20b, 21b, and 22b those for hydrolysis.

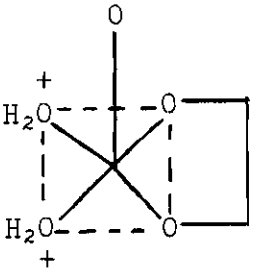
If the transition state is assumed to have the geometry of a trigonal bipyramid, the entering and leaving groups may *a priori* occupy either basal or apical positions but not one of each without violating the law of microscopic reversibility. Structures 20a and 20b represent geometries resulting from the apical attack of the incoming nucleophile. In 20a the ring O-P-O bond angle has been expanded to 120°, and the ring



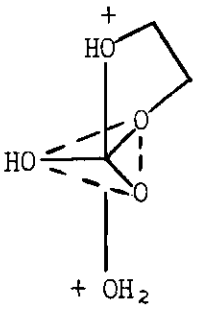
20a



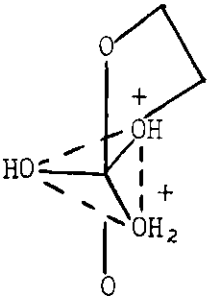
21a



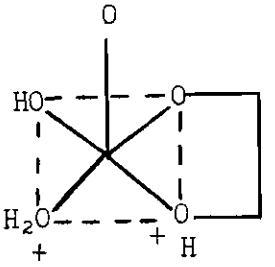
22a



20b



21b

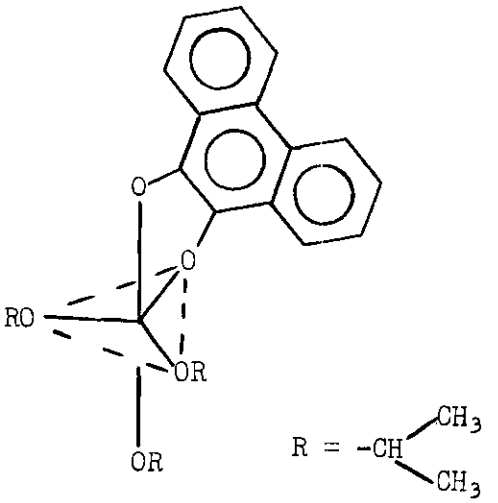


22b

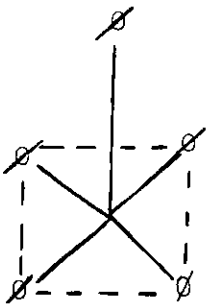
occupies two basal positions; in 20b the O-P-O bond angle is contracted to 90°, and the ring spans one apical and one basal position. These two geometries are quite different and therefore are to be rejected. Significantly, Westheimer's calculations (102) show that an expansion of the ring O-P-O bond angle in a cyclic, pentacoordinate phosphate derivative is accompanied by a substantial increase in the system's strain (3-7 kcal/mole).

Structures 21a and 21b are trigonal bipyramids derived from a basal attack of the incoming nucleophile. In both structures the ring O-P-O bond angle is 90°, and the ring spans one apical and one basal

position. Hence these two geometries are acceptable. Similarly the square pyramidal structures 22a and 22b represent acceptable transition state geometries. Ramirez and coworkers (59) have recently shown by an X-ray structure determination that the pentaalkoxy phosphorane, 23, exists in the form of a trigonal bipyramid with the ring spanning one apical and one basal position. A trigonal bipyramid is also the geometry most commonly associated with the pentahalophosphoranes and their derivatives (97). Thus, a trigonal bipyramidal transition state geometry would appear to be preferred even though pentaphenyl phosphorane, 24, seems to possess the square pyramid structure (106).



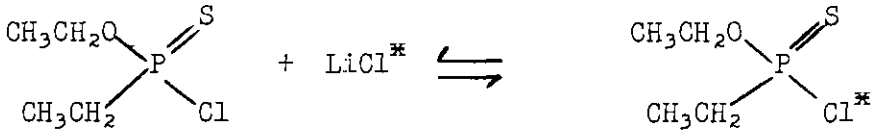
23



24

The trigonal bipyramid structures 21a and 21b predict a stereochemical inversion of configuration in nucleophilic substitutions at phosphorus. The square pyramids 22a and 22b, on the other hand, predict

retention of configuration at phosphorus. Michalski and coworkers, studying a series of optically active phosphonothioates, have recently reported a "Walden inversion" about phosphorus (87). These workers have also shown that inversion accompanies the chloride ion exchange in optically active ethyl ethylphosphonochlorothioate (86) (Eq. 20). Hamer (57) and Green and Hudson (54) have reported inversion resulting from nucleophilic attack at phosphorus in various, substituted phosphonates.



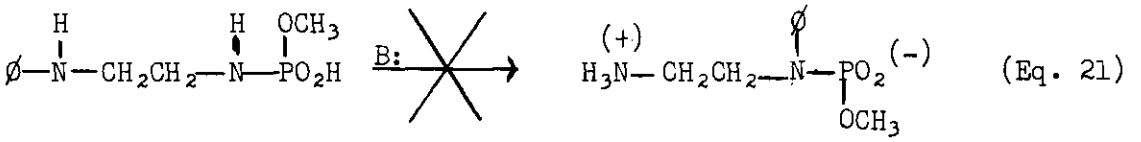
(Eq. 20)

(-) (±)

$$k_{exchange} = 1/2\ k_{racemization}$$

Green and Hudson, in a review of the stereochemistry of nucleophilic attack at phosphorus, also conclude that inversion pathways appear to be predominating processes (53). Though the stereochemistry of nucleophilic attack at phosphorus in cyclic and acyclic phosphate esters has not been studied, inversion of configuration is to be expected, and structures 22a and 22b can probably be eliminated. This position is further supported by the failure of Todd's group to find phosphoryl migration in the base-catalyzed hydrolysis of the ribonucleic acids (19) and by the absence of any intramolecular phosphoryl migration in the reactions of a series of β-aminophosphoramidic acids with 1-naphthylamine (58) (Eq. 21).

If the hydrolysis and exchange reactions proceed through discreet, pentacoordinate intermediates, not transition states, then the



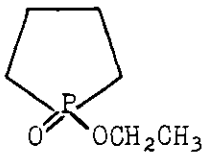
(Eq. 21)

entering and leaving groups may occupy both apical and basal positions without violating the law of microscopic reversibility. (Haake's exchange observations (56), of course, may be interpreted also as evidence for the formation of a stable intermediate.) The pentacoordinate intermediate now represented by structure 20a, however, may still be eliminated on the basis of the calculations by Westheimer's group (102) and the X-ray structure of Ramirez (59). It would also seem unlikely that the entering and leaving groups would occupy both apical and basal positions even though a discreet intermediate may be formed. Certainly the transition states leading to intermediate formation from an apical approach would be energetically quite different from the transition states leading to that same intermediate from the basal approach. Hence, the activation free energies for apical and basal approaches should be quite different, and one process or the other should predominate.

The transition state (or intermediate) structures 21a and 21b are consistent with all that is currently known about the hydrolysis of ethylene phosphate and the intramolecular transesterification reactions studied by Brown and coworkers (17,18,22,23). Westheimer and his students, however, have presented some observations which are not always consistent with the picture given by structures 21a and 21b. The observations are presented briefly in the following paragraph.

Propyl phostonate (14), in sharp contrast to ethylene phosphate, does not undergo prior phosphoryl O^{18} exchange in acid. As already

mentioned methyl propyphostonate hydrolyzes with exclusive ring opening (41); methyl ethylene phosphate hydrolyzes (general base catalysis) with about 70% ring opening and 30% ring retention (31). The five-cyclic phosphinates, 25, 26, and 27, though probably strained (102), hydrolyze in both acid and base at rates comparable to the acyclic phosphinates 28, 29, and 30 (40). Experimental conditions have not been reported.



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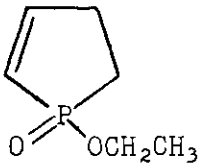

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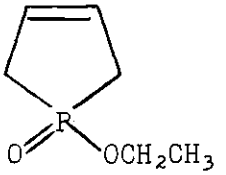

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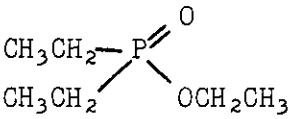

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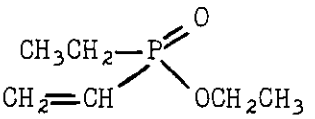

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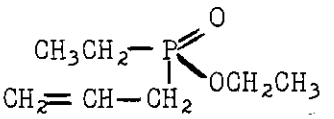
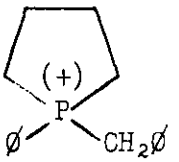
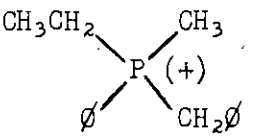


30

By contrast, Wickersham (108) has observed that the hydrolysis of the five-cyclic phosphonium salt, 31, is about 200 times faster than its acyclic analogue, 32 (66).

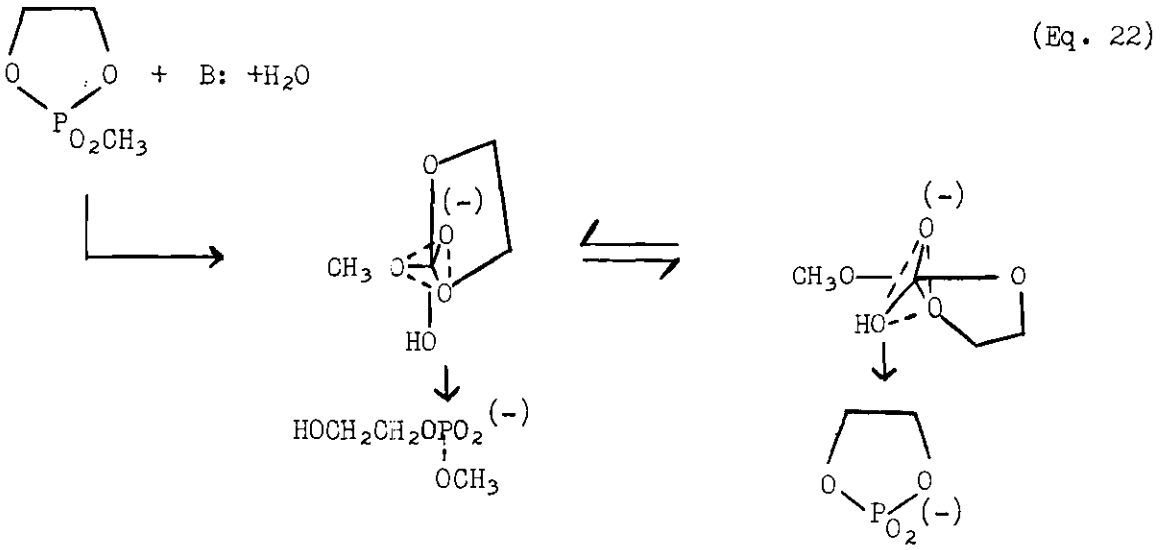


31



32

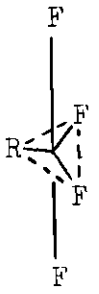
In order to account for the unexpected behavior of the five-cyclic phosphonates and phosphinates, Dennis and Westheimer have proposed that nucleophiles attack phosphorus from the apical position with the subsequent formation, in some systems, of trigonal bipyramidal phosphorane intermediates (41). These intermediates then undergo pseudo-rotation, a process first postulated by Berry (13) to explain the rapid equilibration of the fluorine atoms in the various fluorophosphoranes (97). The loss of the leaving group from the apical position can, of course, yield either product or starting material. The mechanism is illustrated by considering the base-catalyzed hydrolysis of methyl ethylene phosphate (Eq. 22).



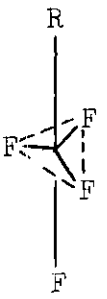
(Eq. 22)

Dennis and Westheimer also assign to the apical positions the more electronegative groups to the exclusion of the less electronegative groups. A significant energy barrier, then, may exist toward replacing a more electronegative group by a less electronegative group in the apical

position. Hence, as observed (97), an alkyl fluorophosphorane, 33, is preferred over its isomeric form, 34.



33



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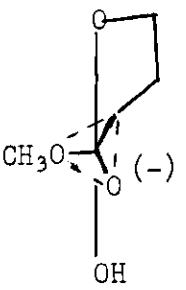
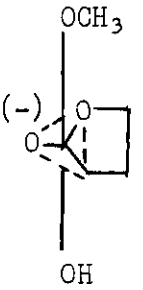
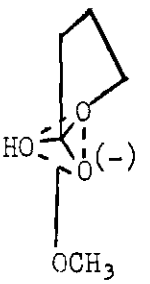


35



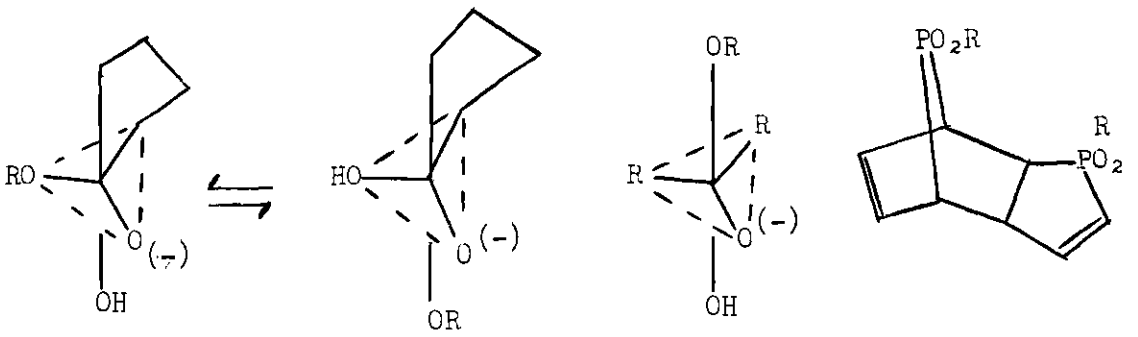
36



37

This mechanism explains the hydrolytic behavior of the five-cyclic methyl propylphosphonate (17). The trigonal bipyramidal intermediate may collapse to either starting material or product by loss from the apical positions of either hydroxide (or water) or of the ring-forming alkoxide (or alcohol), (35). In order to lose methoxide (or methanol), the intermediate, (35), would have to pseudo-rotate. Pseudo-rotation about the methylene group would force the ring to span two basal positions, (36), requiring a C-P-O bond angle of about 120° and would, therefore, introduce considerable strain into the system (59,102); pseudo-rotation about the basal phosphoryl oxygen would force the methylene group into an apical position, (37), which is energetically forbidden (41). Hence, the intermediate, (35), collapses much faster than it can undergo pseudo-rotation. This same argument explains why propylphostonate (14) fails to undergo O^{18} exchange into the phosphoryl group before hydrolysis in acid (46).

This mechanism also explains qualitatively the hydrolytic behavior of the five-cyclic phosphinates (25, 26, and 27) relative to their acyclic analogues (28, 29, and 30). The first trigonal bipyramidal intermediate formed from 28 is represented by formula 38, in which, of course, a methylene group must occupy an apical position. The energy required to generate the intermediate (38) with a methylene group in the apical position is thought to be offset by the relief of ring strain probably present in the five-membered rings (41,102). Hence, the five-cyclic compounds hydrolyze at rates comparable to the acyclic phosphinates where no apical alkyl groups are required in the transition state or intermediate (40). Pseudo-rotation of 38 to 39 followed by the loss of ethoxide (or ethanol) then gives the observed product. It is logically assumed that an intermediate generating a C-P-C ring bond angle of 120° is not formed. Dennis and Westheimer feel that the rapid hydrolysis of one (and

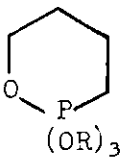


38 39 40 41

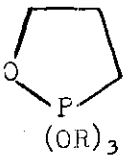
only one) of the ester groups in 41 is an example in which the barrier to placing an alkyl group in an apical position is more than offset by

the relief of strain (41). Experimental details have not been reported.

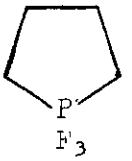
The pseudo-rotation mechanism, of course, requires much testing. Perhaps one of the easiest tests lies in the identification of a discreet intermediate in the alkaline hydrolysis of methyl ethylene phosphate and in both the alkaline and acid hydrolyses of the five-cyclic phosphinates. If intermediates are formed, the structural requirements and experimental conditions necessary for their formation would be of prime importance.* Perhaps the apparently enhanced positive charge on phosphorus in the five-cyclic vs. the six-cyclic and acyclic esters plays an important role in determining intermediate stabilities. If pseudo-rotations do indeed play a part in phosphorus ester hydrolyses, these processes may be observable in mixed phosphoranes such as 42a and 42b. It is interesting that, apparently, the five-cyclic fluorophosphorane, 43a, undergoes pseudo-rotation much faster than the six-cyclic compound, 43b (90,97).



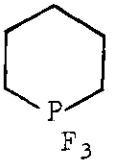
42a



42b



43a



43b

An interesting chemical test of mechanism may emerge from a study of the solvolysis of the very reactive bicyclic triester, 44, recently

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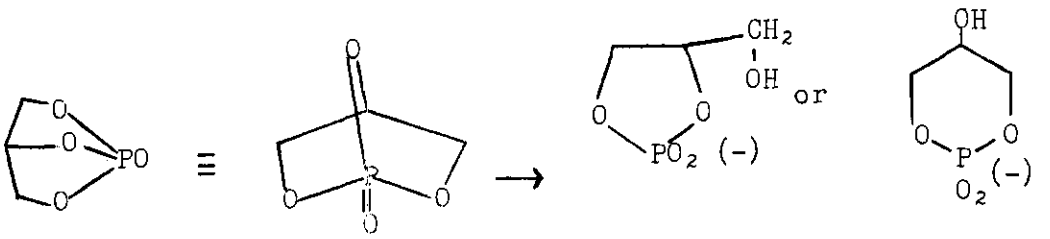
* Haake (56) has argued that the energy required to overcome the coulombic repulsion between the two reactants is too large to allow the formation of a discreet intermediate in the alkaline hydrolysis of ethylene phosphate.

synthesized by Denney and Varga (39). A priori, solvolysis products arising from either basal or apical attack and departure would seem possible. Assuming that in the solvolysis transition state or intermediate each five-membered ester ring prefers to span one basal and one apical



44 45a 45b

position, but not two of each, the initial product derived from basal attack and departure should be the β-hydroxy, five-cyclic 45a. Pseudo-rotation of an apical-attack intermediate would require either that a six-membered ring span two apical positions, or that one five-membered ring span two basal positions. The energy requirements for the formation of two such intermediates would appear to be unreasonably large and, hence, pseudo-rotation of the apical-attack intermediate should be prohibited. The apical-attack mechanism therefore would demand the formation of the β-hydroxy, six-cyclic 45b. The observation of a mixture containing significant amounts of both the initial solvolysis products would only cloud the issue further and be a monstrous event indeed. Of course, the kinetic order and position of bond cleavage should be determined.

The finite possibility that variously substituted phosphorus esters may solvolyze through different transition states cannot be ruled out in the absence of experimental proof. Though the stereochemistry of nucleophilic attack at phosphorus cannot distinguish between the basal

attack (20a and 20b) and the pseudo-rotation mechanisms, it can certainly distinguish between a trigonal bipyramidal and a square pyramidal transition state or intermediate. Westheimer's idea of studying the solvolysis of a bridgehead phosphorus ester is certainly worthy of investigation in this connection (103). The hydrolysis of acyclic tetraalkyl phosphonium salts apparently proceeds with inversion of configuration about phosphorus (66).

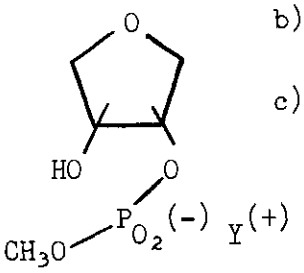
Fukuto and Metcalf have observed that several p-nitrophenyl five-cyclic phosphate triesters hydrolyze extremely rapidly at pH 8.5 to yield only ring-retained products (51). The mechanisms of these reactions have not been studied. Methyl ethylene (31) and methyl pinacol phosphates yield both ring-retained and ring-opened products during general base and hydroxide ion-catalyzed hydrolyses, respectively. In aqueous base, however, methyl ethylene phosphate apparently yields almost exclusively ring-opened opened product (35,71). These observations may be readily explained in terms of either the transition state (or intermediate) structure, 20b, or the pseudo-rotation mechanism, since the product-determining step in all cases is probably a sensitive function of the nature of the leaving group. In this regard, the hydrolysis products of the fully esterified five-cyclic phenyl and p-nitrophenyl phosphonates would be of extreme interest. Detailed studies of the hydroxide ion-catalyzed as well as the neutral and acid hydrolyses of the five-cyclic triesters have not been reported. Newton, Cox, and Bertrand do report, however, that methyl pinacol phosphate solvolyzes in water to give only methanol and pinacol phosphoric acid (92). Also, t-butyl pinacol phosphate solvolyzes (probably by an S_N1 process) to give only the ring-retained

pinacol phosphoric acid (91) Much more work is needed to understand the chemistry of these most interesting types of compounds

Description of the Research

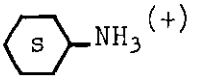
A quantitative energy approach to the hydrolyses of the five-cyclic phosphates has been neglected. Therefore, the activation energies for both the acid- and base-catalyzed hydrolyses of ethylene phosphate have been measured. Though these two sets of data are of very limited importance when taken separately, they take on some significance when compared to each other and the few other activation data available (32,46).

All previously studied ribonucleic acid models have suffered from the lack of a five-cyclic sugar ring skeleton. In order to evaluate the structural importance of the ribose ring itself, the diesters 46 and 47 have been prepared and their hydrolysis rates studied. The study of 46 and its prototypes is of particular importance in the search for the re-

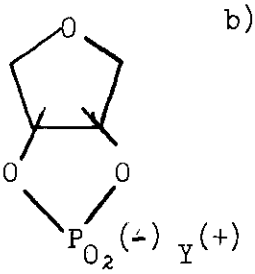


46

a) $Y^{(+)} = H^{(+)}$

b) $Y^{(+)} =$  $NH_3^{(+)}$

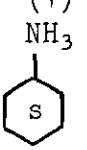
c) $H^{(+)}$ = Quinine $H^{(+)}$



47

a) $Y^{(+)} = H^{+}$

b) $Y^{(+)}$ =

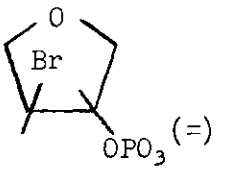


quirements of general base, general acid, and enzymatic catalysis in phosphate ester systems. This is also a good comparison model for the (at least partial) evaluation of the transesterification "entropy boost"

built into the molecule structurally through the rigidly-held β-hydroxyl group. Since equilibrium alkoxide ion formation (22) is a consideration in a study of the transesterification rates and activation parameters (in particular, activation entropies), the relative rates and activation parameters (especially activation entropies) for the intramolecular cyclization of 48 and 49 have been studied. An entropy "boost" is expected in 49 relative to 48 though, of course, a quantitative extension of the magnitude of such a "boost" to the transesterification reactions is not valid.

$BrCH_2CH_2OPO_3^{(=)}$

48



49

CHAPTER II

EXPERIMENTAL

Instrumentation

Infrared (IR) spectra were recorded on a Perkin-Elmer Infracord Spectrophotometer and were calibrated against the 6.238 μ band of polystyrene. Nuclear magnetic resonance (n.m.r.) spectra were recorded on a Varian A-60 spectrometer and were calibrated against tetramethylsilane (TMS) internal or external standard. Optical rotations were measured on a Bendix automatic recording polarimeter at a wavelength of 546.1 mμ (Hg green). All pH measurements were made on a Beckman Model G pH meter calibrated against standard buffers. All melting points are uncorrected.

Materials

Methanol was dried by distillation from magnesium methoxide (107). Diethyl ether was predried over calcium hydride and then distilled from lithium aluminum hydride immediately before use. Cyclohexylamine, 1-butanol, acetone, phosphorus oxychloride, trimethyl phosphite, and benzene were distilled before using. Dry acetone was prepared by distillation from phosphorus pentoxide. All other common solvents and materials were used without further purification. Distilled or deionized water was used to prepare all aqueous solutions. Deuterated solvents were obtained from Diaprep Incorporated.

Quinine (free base) was purchased from the Fisher Scientific

Company. The 2-buten-1,4-diol was purchased from the General Aniline and Film Corporation. Ribonnuclease was purchased from the Calbiochem Company; the solid was kept refrigerated (but not frozen) at all times prior to use. Cyclohexylammonium pinacol cyclic phosphate was kindly donated by Dr. M. G. Newton. Barium trans-3-bromotetrahydrofuranyl-4-phosphate (49) was kindly donated by Mr. J. J. Farmer (48). Both bromohydrin phosphates (48 and 49) were purified by recrystallization from aqueous ethanol. The esters (barium salts) were dissolved in the minimum amount of water and filtered. The addition of one part ethanol to two parts of the ester solution (V/V) resulted in the formation of flat platelets which, after two such recrystallizations, were colorless.

Barium 2-Bromoethyl Phosphate (48)

The acyclic bromohydrin phosphate was prepared from 2-bromoethanol and phosphorus oxychloride by the method of Kumamoto, Cox, and Westheimer (77) as amended by Cox (32).

Barium Ethylene Phosphate (12)

Ethylene phosphate was prepared by the cyclization of barium 2-bromoethyl phosphate (77). Cyclohexylammonium ethylene phosphate was prepared by the metathesis reaction of cyclohexylammonium sulfate and barium ethylene phosphate (77).

Dinitrogen Tetroxide

The procedure for the preparation of dinitrogen tetroxide from nitric oxide and oxygen, suggested by Dr. J. D. Ray, has been described by Newton (91). An evacuated gas manifold and an evacuated 12-liter flask were filled with nitric oxide to a pressure of slightly less than one atmosphere. Oxygen gas was then introduced slowly into the system

through another part of the manifold. Addition was continued until the pressure of the system ceased to drop. At this stage the beautiful, red dinitrogen tetroxide gas filled the system. One neck of a two-neck collection flask, immersed in either liquid nitrogen or a dry ice-acetone mixture, was attached to the manifold system. The other neck was connected to a vacuum pump. The dinitrogen tetroxide was subsequently trapped in the cold collection flask where it was stored for future use. All connections except the one to the vacuum pump were made with ground glass joints.

meso-Erythritol and cis-3,4-Tetrahydrofurandiol

meso-Erythritol was prepared by the osmium tetroxide-catalyzed hydroxylation of 2-buten-1,4-diol, according to Reppe et al. (95). The acid-catalyzed dehydration of meso-erythritol yielded cis-3,4-tetrahydrofurandiol (29,63). The n.m.r. and IR spectra have been reported by Cleveland (29).

cis-3,4-Tetrahydrofurandiol Cyclic Phosphoric Acid (Carré's Acid 47a)

One hundred six and eight tenths grams (1.03 mole) of cis-3,4-tetrahydrofurandiol and 127.8 grams (1.0 mole) of trimethyl phosphite were mixed with shaking in a stoppered, 500-ml., round-bottom flask. Heat was evolved immediately, and phosphite polymer crystals began to appear after about 15 minutes. Crystallization was complete after several hours. The reaction mixture was then placed under vacuum for 18 hours to remove the methanol formed in the reaction and any unreacted trimethyl phosphite. The polymer was used without further purification; m.p. 100°, increasing with heating. The polymer does not distil at pressures as

low as 0.04 mm.

The polymer was dissolved in chloroform and oxidized with dinitrogen tetroxide by the method of Cox and Westheimer (36). When an excess of dinitrogen tetroxide had been added, the oxidation was halted and the red solution allowed to stand at room temperature exposed to atmospheric moisture. Crystallization of Carré's acid began almost immediately and continued for two days. The acid crystals were then filtered, washed with chloroform, and dried under vacuum.

Yield: 92 g; m.p. 205-210°, lit. (26) 205°.

The IR spectrum shows an acidic hydroxyl group at 4.16 μ, phosphate ester peaks at 8.00, 8.29, 9.00, 9.16, 9.60, and 9.82 μ, and other major peaks at 10.60, 10.90, 11.32, 11.72, 12.40, and 12.75 μ. The n.m.r. spectrum, which is very similar to those of other cyclic diesters of cis-3,4-tetrahydrofurandiol (29), is given in Figure 2. Both the n.m.r. and IR spectra are identical to those of an authentic sample (26).

Carré's Salt (47b)

Twenty-four and nine tenths grams (0.15 mole) of Carré's acid (47a) and a 100 per cent molar excess of cyclohexylamine were triturated together. The tan solid was filtered (suction) and washed with dry ethyl ether. The product was dissolved in dry 1-butanol at room temperature. Addition of dry ethyl ether to the butanol solution caused precipitation in a crystalline state. After two such recrystallizations the product was considered pure.

Yield: 25.7 g. (65%), m.p. 218.5-220.5°.

The n.m.r. (D_2O) and IR (nujol) spectra are given in Figures 3 and 4, respectively. These spectra are identical to those of an authentic

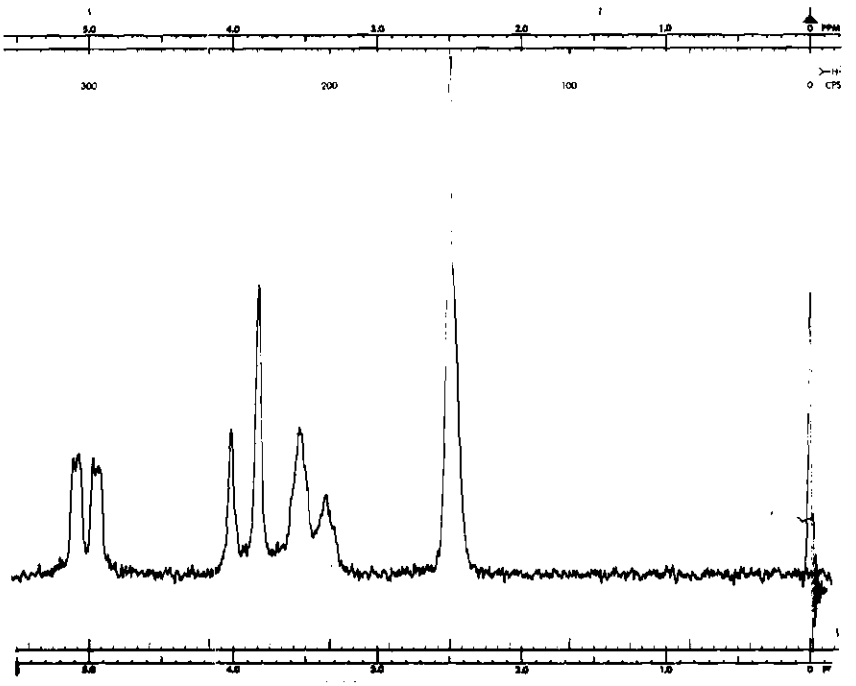


Figure 2. The n.m.r. Spectrum (DMSO-d_6) of Carré's Acid (47a) Relative to TMS External.

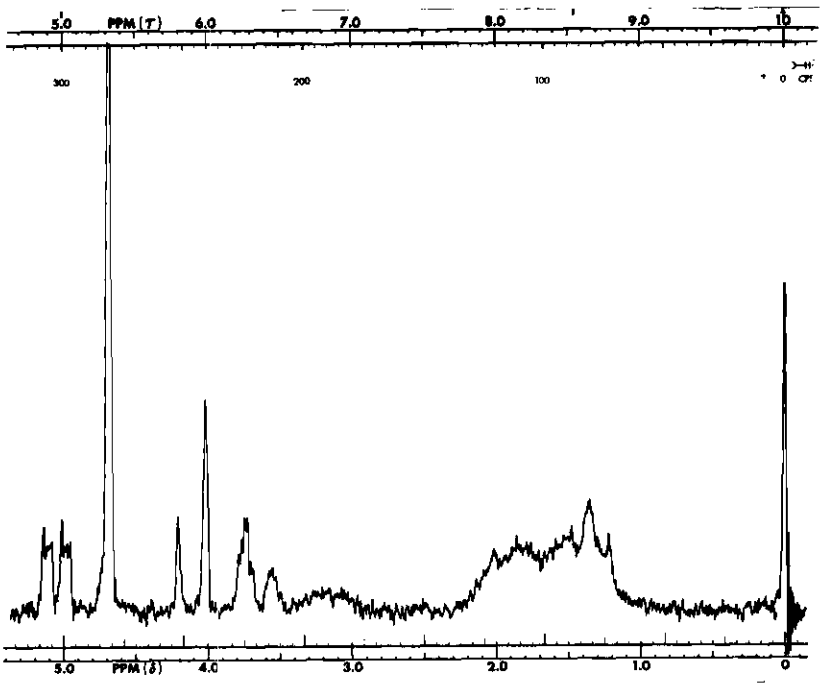


Figure 3. The n.m.r. Spectrum (D_2O) of Carré's Salt (47b) Relative to TMS External.

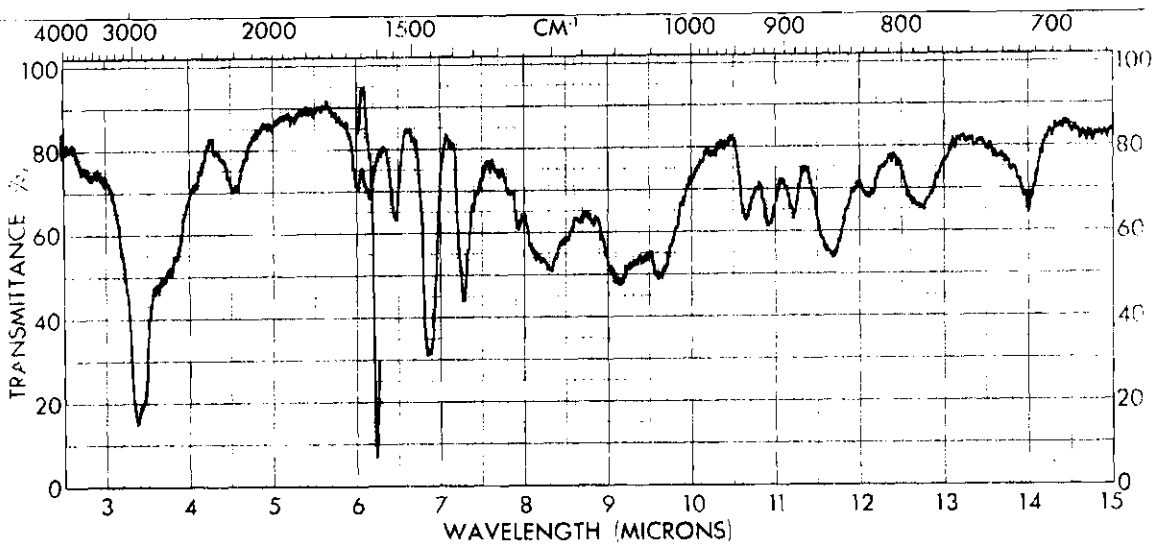


Figure 4. The IR Spectrum (Nujol) of Carré's Salt (47b).

sample prepared by Mr. J. J. Farmer from the cyclization of 49 (48).

Cyclohexylammonium Methyl cis-3-Hydroxytetrahydrofuranyl-4-Phosphate (46b)

To one milliliter of dry methanol in a stoppered flask was added 53 mg. (0.37 mmole) of 47b. The mixture was warmed gently until complete solution had been effected. To this solution was added 37 mg. (0.37 mmole) of cyclohexylamine in one ml. of dry methanol. The very slightly basic solution which resulted was stripped, and the crude product dried under vacuum. Purification was accomplished by recrystallization from 1-butanol and ethyl ether in the manner described for 47b. Yield: 40 mg. (75%), m.p. 144°, authentic sample (55) 147-151.5°.

The n.m.r. (D_2O) and IR (nujol) spectra are given in Figures 5 and 6, respectively. These spectra are identical to those obtained from an authentic sample which has been prepared by the partial hydrolysis

of methyl cis-3,4-tetrahydrofuranyl cyclic phosphate (55).

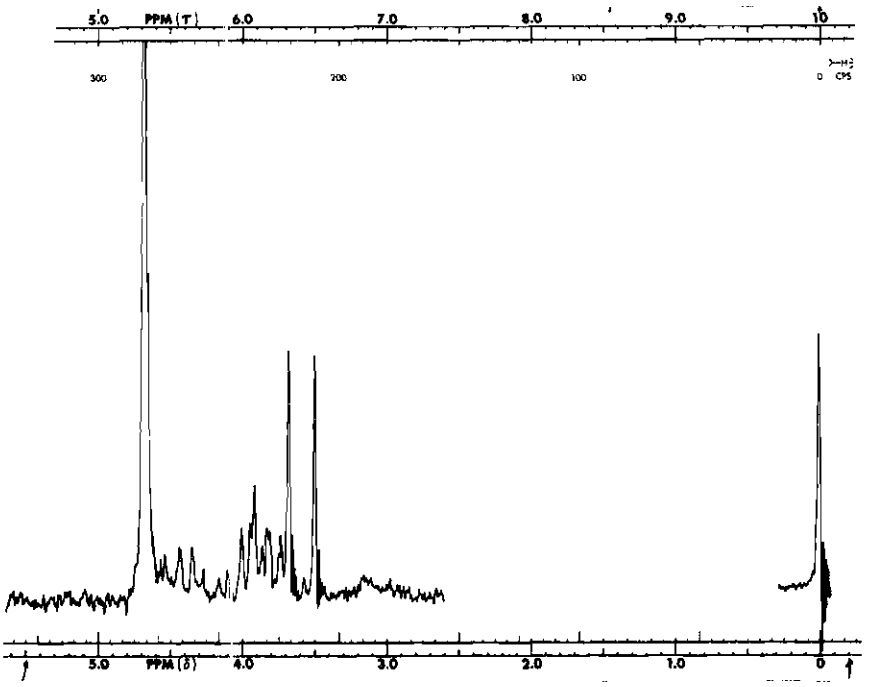


Figure 5. The n.m.r Spectrum (D_2O) of the Cyclohexylammonium Salt (46b) Relative to TMS External.

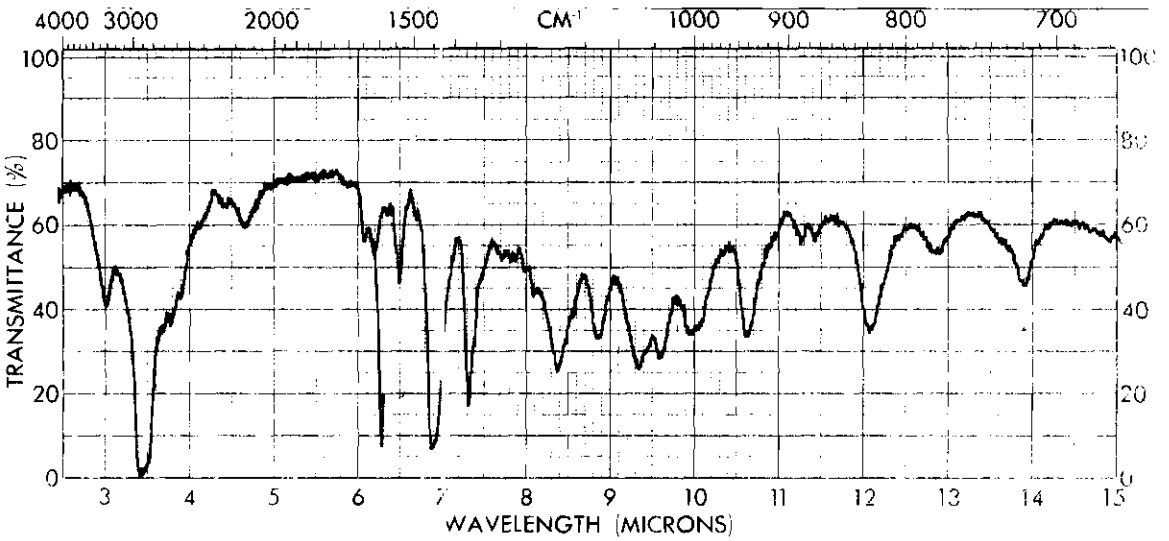


Figure 6. The IR Spectrum (Nujol) of the Cyclohexylammonium Salt (46b).

Quininium Methyl cis-3-Hydroxytetrahydrofuranyl-4-Phosphate (46c)

In one typical experiment 50 ml. of dry methanol was distilled into a 500-ml. round-bottom flask containing 16.9 g. (0.102 mole) of 47a. The flask was tightly stoppered and the mixture warmed gently to effect solution. Then 33.1 g. (0.102 mole) of quinine in 200 ml. of dry methanol was added as rapidly as possible. The very slightly alkaline solution which resulted was stripped and dried. The light red amorphous solid was purified by repeated fractional recrystallizations from dry acetone.

Yield: 8.5 g. (16%), m.p. 150-158° dec.

The ester portions of the n.m.r. spectra of 46b and 46c (D_2O) are identical. In addition, the n.m.r. spectrum of the quinine salt shows complex peaks in the aromatic and aliphatic regions and also an Ar-O-$\underline{CH_3}$ singlet at 3.9 ppm downfield from TMS external. The IR spectrum (nujol) of 46c is given in Figure 7.

Purification of the quinine salt (46c) by fractional recrystallizations from dry acetone proved to be a tedious and unsatisfactory procedure, due in part to the facile decomposition of the quininium cation on exposure to heat and light. In addition, there was formed in several preparations an unidentified, acetone-insoluble product. This product might have been formed in either the initial solvolysis reaction of 47a (partial ring retention upon dissolution into methanol or hydrolysis by residual water not removed from the starting acid or the methanol) or in the purification procedure. This matter was not investigated further. It was subsequently observed, however, that the quinine salt could be both purified chemically and partially resolved by recrystallization of

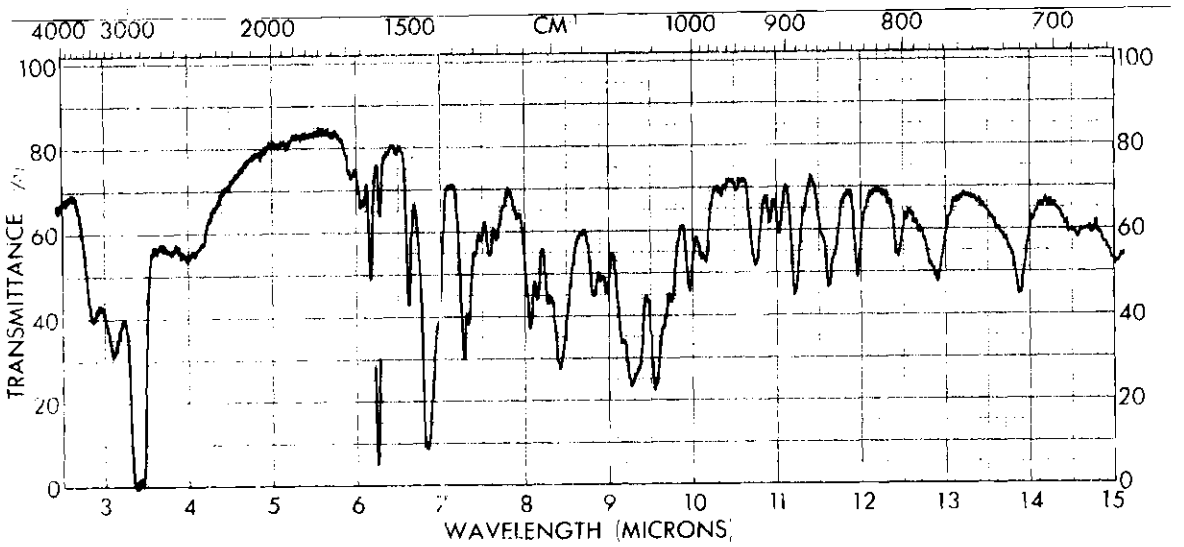


Figure 7. The IR Spectrum (Nujol) of the Quinine Salt (46c).

the crude salt directly from 10 per cent aqueous acetone (V/V). (See below.)

The Resolution of 46c

The quinine salt (46c) was resolved by successive recrystallizations from 10 per cent aqueous acetone (V/V). In a typical experiment, 3.0 g. was recrystallized once from 12 ml. of solvent and subsequently six times, each from 10 ml. of solvent. At this point 0.99 g. of salt were obtained; m.p. 163.5-164.5°. After conversion into the cyclohexylammonium salt (46b), $[\alpha]^{25}_{546.1}$ = +11.5 (EtOH), -3.2 (H_2O). Further recrystallizations of 46c did not lead to a change in the melting point or specific rotation of 46b.

The specific rotation of the quinine salt after a given number of recrystallizations was not indicative of the progress of the resolution. In one experiment, the quinine salt (as fine needles) showed a specific rotation of -143 (EtOH) after five recrystallizations from 10 per cent aqueous acetone. In another experiment, however, the salt (a fluffy solid) showed a specific rotation of -170 (EtOH) after five recrystallizations. Both samples were dried under vacuum before each rotation was taken, and indeed, conversion of both samples into 46b gave a sample with a specific rotation of +11 (EtOH). The specific rotation of unresolved 46c was -152 (EtOH).

The second isomer (levo in EtOH) was obtained (70 per cent enrichment) by working with the mother liquor fractions in the experiment described above. All mother liquor fractions were combined and stripped. The resulting, brown solid was then recrystallized, the mother liquor fraction being retained and stripped. Five such cycles yielded 0.7 g. of the enriched salt which, upon conversion to 46b, showed an $[\alpha]^{25}_{546.1}$ = -8 (EtOH), $[\alpha]^{40}_{546.1}$ = +1.8 (H_2O).

Conversion of 46c into 46b

The experiment described below is demonstrative of the general procedure employed for the conversion of both isomers. To 1.5 ml. of warm 1-butanol was added with stirring 0.32 g. of 46c and 16 drops (0.19g.) of cyclohexylamine. The addition of 18 ml. of dry ether followed by cooling yielded fine needles which were collected and recrystallized again from one ml. of 1-butanol, one drop of cyclohexylamine, and 20 ml. of dry ether. The fine needles were collected, washed with dry ether, and dried under vacuum. Yield: 0.15 g. (89%), m.p. 139.5-141°.

For the second isomer (levo in EtOH), m.p. 140.5-141.5°. Mixed m.p. 143-147°. Pure, racemic 46b m.p. 147-151.5°.

Attempted Enzymatic Cyclization and Hydrolysis of 46b and 47b, Respectively

To approximately 20 per cent aqueous (D_2O) solutions each of 46b and 47b in n.m.r. spectral tubes was added about 10 milligrams of ribonuclease. The two n.m.r. spectra were then immediately recorded and observed to be identical to those given in Figures 5 and 3. The n.m.r. spectra of these two samples were subsequently reexamined at various time intervals. After a period of seven months at room temperature there was no evidence of any reaction of either 46b or 47b.

Kinetic Methods

All temperatures were measured on a thermometer calibrated by the National Bureau of Standards. Sodium hydroxide solutions were prepared by diluting standard volumetric solutions purchased from the Anachemia Chemical Company. These solutions were then standardized against potassium hydrogen phthalate primary standard. The sodium deuteroxide solution was prepared by treating 6.70 g. of Dri-Na (a sodium-lead alloy purchased from the Baker Chemical Company) with heavy water. After gas evolution had ceased, the liquid was filtered through glass wool and brought to 100 ml. in a volumetric flask. This solution was then standardized against potassium acid phthalate. The solutions for the barium ion catalysis studies were prepared by dissolving a weighed amount of barium chloride in the proper volume of sodium hydroxide solution. Acid solutions were prepared by diluting weighed amounts of 70 per cent perchloric acid. Each acid solution was standardized by

titration against a standard base solution. All ionic strengths were maintained with sodium perchlorate.

The kinetics of the alkaline hydrolysis of 46b were measured by following the change in optical rotation vs. time. Temperatures were maintained constant within 0.1°. A weighed amount of ester was placed directly into a glass, water-jacketed cell (volume 7.0; l 0.5 dm). The cell was brought to temperature and the base solution, also at the correct temperature, was added as rapidly as possible. The cell was then stoppered and placed into the polarimeter. The kinetics of the alkaline hydrolysis of 12 and 47b were measured by a titration technique similar to that of Kumamoto, Cox, and Westheimer (77). Aliquot samples were withdrawn from the polyethylene container* at given times with a Cornwall syringe and quenched to pH 3.0-3.5 with 0.1 M perchloric acid. Each sample was degassed with nitrogen and then titrated with a 0.01 M sodium hydroxide solution. The quantity of this solution (ml) required to raise the pH from about 4.2 to 8.3 was taken as a measure of the phosphate monoester (i.e., hydrolysis product) in each sample. The kinetics of the acid-catalyzed hydrolysis of 12 were followed in the same manner except that each sample was quenched with about 0.1 M base, not acid. Temperatures were maintained constant within 0.05°.

Since the hydroxide ion concentration was always present in a large excess, pseudo first-order rate constants were measured directly by plotting log $(X_{\infty} - X_t)$ vs. t, where X was α or ml. Good straight lines were obtained in all cases. The true second-order rate constants were

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* Previously boiled in concentrated, aqueous base.

calculated from the pseudo values. The pseudo rate constants for the racemization of 46b were measured through at least two half-times, those for the hydrolysis of 47b and 12 through at least one and one-half half times. In all rate studies in base the cyclohexylammonium salts of the esters were used in concentrations of about 0.02 M. Barium ethylene phosphate (≈0.01 M) was used for the acidic kinetic studies where pseudo first-order constants were obtained. The constants, of course, were a sensitive function of the acid concentration employed. Kumamoto, Cox, and Westheimer (77) showed that the monoester, 2-hydroxyethyl phosphate, was the product of the hydrolysis of ethylene phosphate. That the monoester, cis-3-hydroxytetrahydrofuranyl-4-phosphate, was the final hydrolysis product of both 46 and 47 was demonstrated by Guida (55). Predictably, no epoxide formation was observed.

Tables 1, 2, and 3 present some typical data for the alkaline hydrolyses of 46b, 47b, and 12, respectively. These data are plotted in the accompanying Figures 8, 9, and 10.

Cox (32,77) and Farmer (48) demonstrated that the products of the cyclizations of the bromohydrin phosphates (48 and 49) were the five-cyclic ethylene phosphate (12) and cis-3,4-tetrahydrofuranyl cyclic phosphate (47). The kinetics of these reactions were followed by potentiometric titrations of the liberated bromide ion with a 0.01 M silver nitrate solution. The titrations were performed with a Beckman Model G pH meter equipped with saturated calomel and silver electrodes. The endpoints fell at about +230 millivolts (mv) although the exact value of the end-point potential appeared to be a function of the activity of the silver electrode. The procedure followed was roughly the same

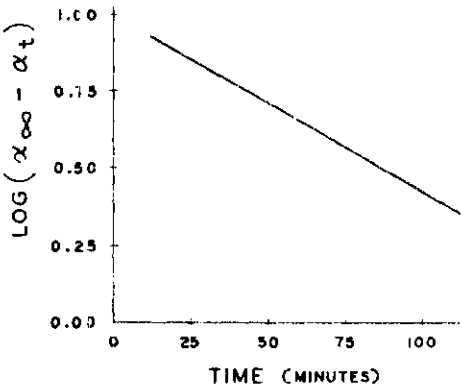

Figure 8. A Plot of the Data in Table 1.

Table 1. Rate Data for the Racemization of 46b in 0.101 M Base at 39.7° and Ionic Strength 0.50.

t, min.	α_t	$\alpha_\infty - \alpha_t$	$\log(\alpha_\infty - \alpha_t)$
15.0	5.90	8.00	0.903
22.5	6.50	7.40	0.869
30.0	7.20	6.70	0.826
37.5	7.80	6.10	0.785
45.0	8.40	5.50	0.740
52.5	8.90	5.00	0.699
60.0	9.35	4.55	0.658
67.5	9.85	4.05	0.608
75.0	10.21	3.70	0.568
82.5	10.55	3.35	0.525
90.0	10.80	3.05	0.484
97.5	11.20	2.70	0.431
105.0	11.50	2.40	0.380
∞	14.90	----	-----

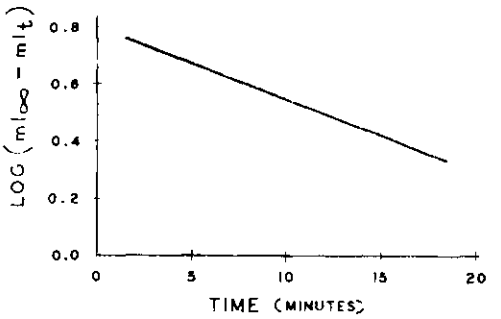

Figure 9. A Plot of the Data in Table 2.

Table 2. Rate Data for the Hydrolysis of 47b in 0.281 M Base at 49.7° and Ionic Strength 0.30.

t, min.	ml_t	$ml_\infty - ml_t$	$\log(ml_\infty - ml_t)$
2.00	1.31	5.53	0.743
4.00	1.83	5.01	0.700
6.00	2.36	4.48	0.651
8.10	2.93	3.91	0.592
10.0	3.33	3.51	0.545
12.0	3.73	3.11	0.493
13.9	4.03	2.81	0.449
16.1	4.38	2.46	0.391
18.0	4.63	2.21	0.344
∞	6.84	----	-----

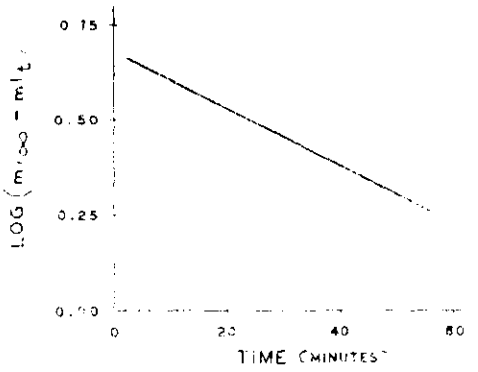

Figure 10. A plot of the Data in Table 3.

Table 3. Rate Data for the Hydrolysis of 12 in 0.283 M Base at 39.7° and Ionic Strength 0.30.

t, min.	ml_t	$ml_\infty - ml_t$	$\log(ml_\infty - ml_t)$
5.00	1.61	4.47	0.650
10.0	2.01	4.07	0.610
15.9	2.43	3.65	0.562
20.0	2.71	3.37	0.528
25.0	2.98	3.10	0.491
30.0	3.22	2.86	0.456
38.0	3.61	2.47	0.393
44.0	3.85	2.23	0.348
50.0	4.05	2.03	0.308
∞	6.08	----	-----

as that used in following the alkaline hydrolyses of 47b and 12. Aliquots (≈ 3 ml) were withdrawn from the polyethylene bottle* at different times with a Cornwall syringe and quenched with about 3 ml of 2 M perchloric acid. Each aliquot sample was then carefully titrated to the end point. The volume (ml) of the 0.01 M silver nitrate solution required to reach the end point was taken as a precise measure of the amount of product present at any time. Plots of $\log(ml_\infty - ml_t)$ vs. t gave good straight lines proving both cyclization reactions to be first-order processes. Each reaction was followed through about one and one-half half times. Since the cyclization of 49 was relatively slow, the infinity values (ml_∞) were obtained by heating about 15 ml of the reaction solution in a sealed tube; the heating was performed for a minimum of ten half-times. These solutions, of course, were equilibrated at the correct temperature before the infinity aliquot samples were taken. As long as the vapor pressure above the kinetic solution was allowed to equilibrate with atmospheric pressure before the bottle was tightly sealed, solvent loss was insignificant, even at 76°. Temperatures were maintained constant within 0.05°.

Since hydrolysis of the two phosphate monoesters (48 and 49) can occur (Eq. 23), the cyclization rates were measured in a borate buffer solution (pH 9) prepared by dissolving 12.587 g (0.033 mole) of disodium

$$ROPO_3^{(=)} + H_2O \underset{}{\overset{K_h}{\rightleftharpoons}} ROPO_3H^{(-)} + {}^{(-)}OH \qquad \text{(Eq. 23)}$$

$$K_h = 10^{-7}$$

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* Previously boiled in concentrated aqueous base.

metaborate decahydrate in one liter of deionized water. At this pH essentially all of each monoester was present as its dianion. Temperature effects on the pH of the solution were neglected (see reference 11 for a tabulation of the temperature effects on the pH of buffer solutions). Ester concentrations of 0.01 - 0.02 M were used; the barium salt was studied in all cases. An ionic strength of 0.20 or 0.60 was maintained with sodium perchlorate.

Titrations of acidified blank samples containing only borate, borate and added sodium bromide, and borate, added sodium bromide, and added, unreacted starting esters showed that boric acid and the unreacted, protonated starting esters had no effect on the bromide determinations. The titration curves obtained from the analyses of the kinetic samples always showed two breaks, one at about +230 mv (bromide) and another at about +350 mv. The separation between the two breaks was small, initially (0.2 ml. of 0.0. M silver nitrate solution) and increased as the run progressed (0.5 ml. after ten half-times); the rate of increase was very slow relative to the rate of bromide ion elimination. That the second break also occurred when an acidified sample of bromide and barium 2-hydroxyethyl phosphate (prepared *in situ* from barium ethylene phosphate) was titrated suggested that this second break was the result of a phosphate-silver precipitate or complex. Since the material(s) responsible for the second break had no effect on the precision of the bromide determinations, this matter was not investigated further.

Table 4 gives some typical data for the cyclization of 48; these data are plotted in Figure 11.

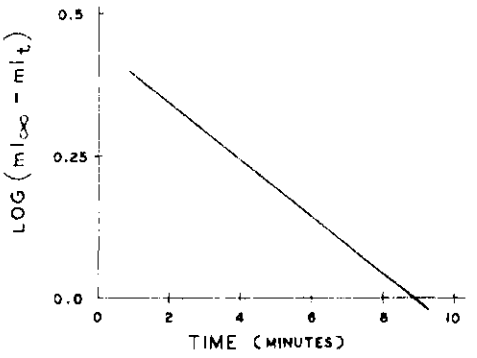


Figure 11. A Plot of the Data in Table 4.

Table 4. Rate Data for the Cyclization of 48 in a 0.033 M Borax Buffer at 61.8° and Ionic Strength 0.20.

t, min.	ml_t	$ml_\infty - ml_t$	log $(ml_\infty - ml_t)$
1.00	0.34	2.49	0.396
2.00	0.62	2.21	0.344
2.98	0.88	1.95	0.290
4.00	1.07	1.76	0.246
6.00	1.45	1.38	0.140
7.00	1.59	1.24	0.093
8.00	1.72	1.11	0.045
9.00	1.85	0.98	-0.009
∞	2.83	-----	-----

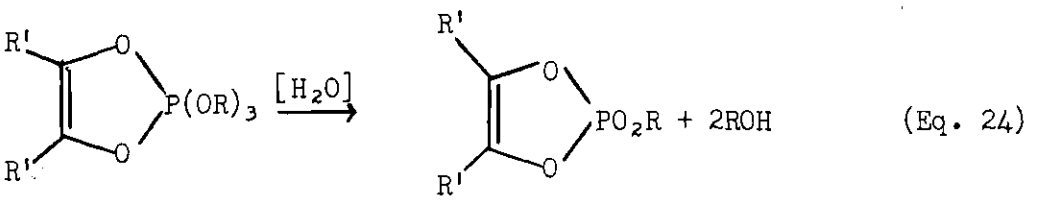
CHAPTER III

RESULTS

Syntheses

The syntheses of five-cyclic phosphates have been accomplished by a variety of means. The cyclization of 2-bromoethylphosphate (77) or 2-chloroethyl phosphate (80) produces ethylene phosphate. Similarly, a cyclization of trans-3-bromotetrahydrofuranyl-4-phosphate produces salts of 47a (48). "Activated" β-hydroxy diesters have also been used to prepare the cyclic moieties (74). Many 1,2-diols yield five-cyclic esters upon reaction with tri- and tetravalent phosphorus derivatives. Carré's (26) quaint preparation of 47a from meso-erythitol and 85 per cent phosphoric acid has been mentioned in the experimental section. Normally, however, the 1,2-diols do not yield five-cyclic esters on reaction with phosphoryl derivatives such as the phosphorus oxychlorides or trialkyl phosphates (100). Reaction with trivalent phosphorus compounds, the phosphorus trichlorides, triphenyl phosphite, and the like, often produces in good yield the five-cyclic phosphites (2,83) which are readily converted into the corresponding phosphates (32). The preparations of five-cyclic esters by the hydrolyses of the five-cyclic phosphoranes (Eq. 24) have been reported by Ramirez (94). These fascinating reactions have received but little study to date.

Brown et al., (22,23) have described the preparation of many β-hydroxy phosphates employing the reactions of monoesters with epoxides. Cox (32) has observed that methyl ethylene phosphate readily undergoes

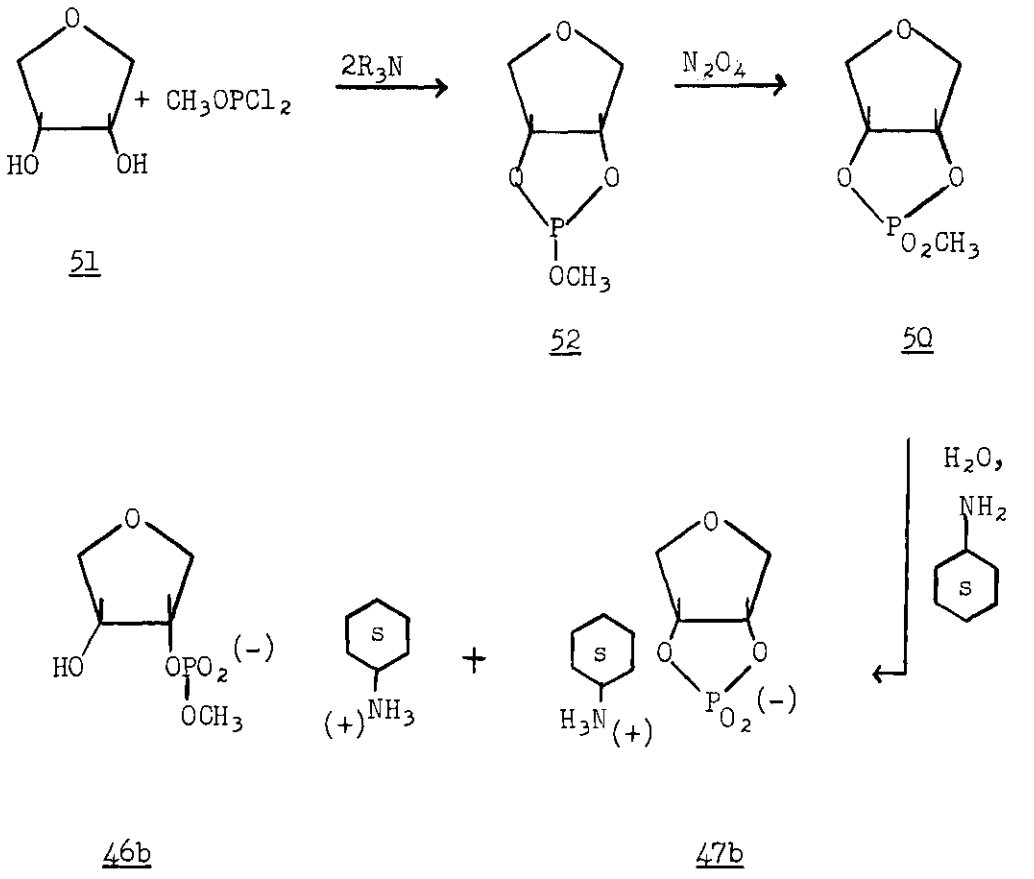


(Eq. 24)

solvolysis in dry methanol, producing dimethyl 2-hydroxyethyl phosphate. Ring opening reactions of this type are probably quite general in nature and can be used for preparative purposes. Kosalapoff (75) and Khorana (74) give many other useful syntheses for phosphate esters.

Though the discovery of general phosphate syntheses is of interest, the immediate problem facing us was a good preparation of the β-hydroxy diester (46). Cox, and subsequently Guida (55), succeeded in preparing this ester by the partial hydrolysis of 50. Their route is presented in Figure 12. This synthesis of 46b was successful although the overall yield was sometimes quite low and the product was often contaminated by 47b. Furthermore, the preparation of the phosphite, 52, was a tedious job indeed.

Hoping primarily to circumvent the difficulty in preparing 52, the cis-3,4-tetrahydrofurandiol, 51, was treated with trimethyl phosphite in the absence of solvent. Rather than 52, a white, crystalline oligomer or polymer was formed. Though the oligomer or polymer was extremely sensitive to moisture, it resisted reaction with dry methanol containing methoxide anion. As expected, the material was readily oxidized by dinitrogen tetroxide (36) an oxidized oligomer or polymer being the product. When the oxidized material (in chloroform) was allowed to



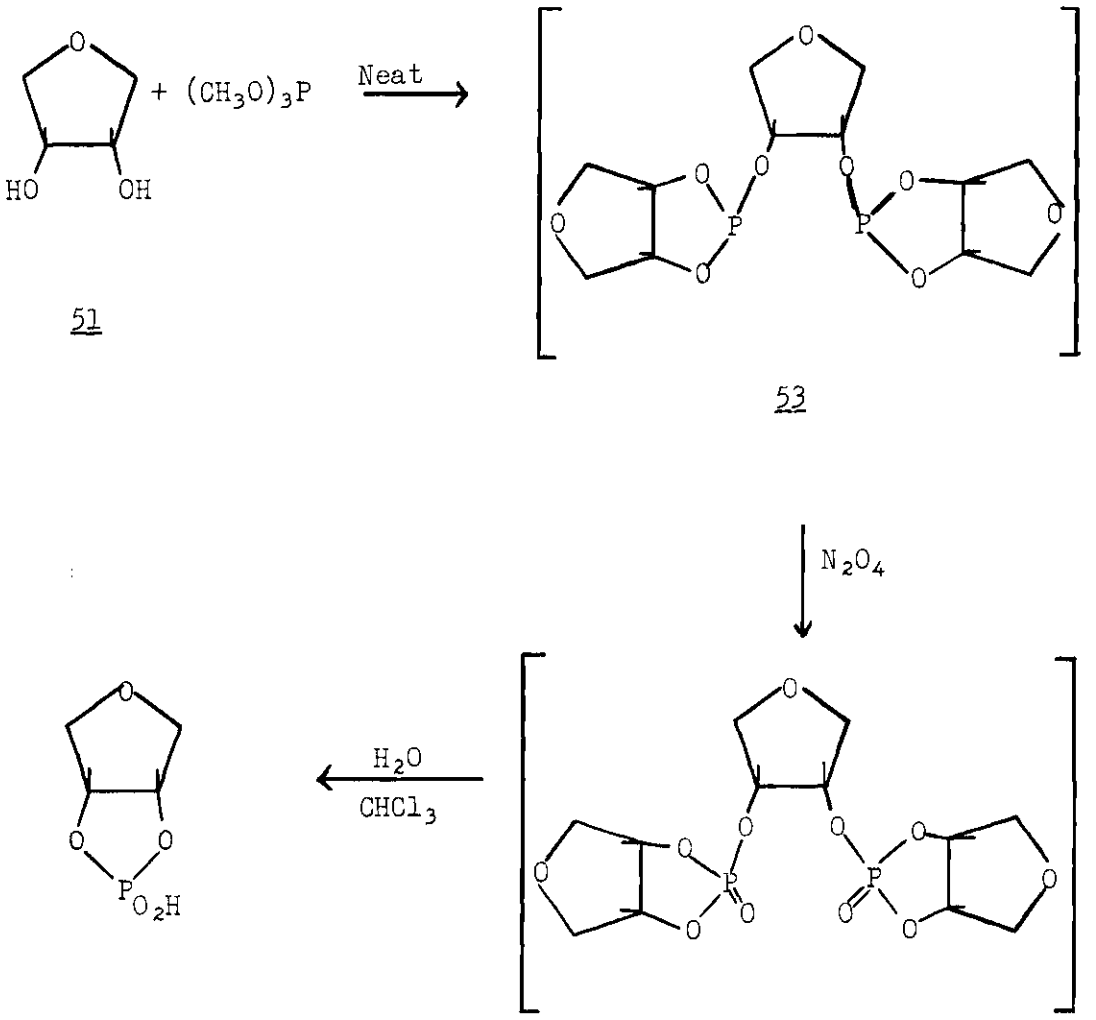
51 52 50

46b 47b

Figure 12. Cox's Preparation of 46b.

sit exposed to atmospheric moisture, beautiful, white crystals of Carré's acid (47a) crystallized from the solution! Carré's acid and its salts were also available both from the synthesis of Carré (26) and Farmer (48). The material obtained from Carré's methods, though suitable enough, was often difficult to purify. In addition, in our hands the yields ranged from about zero to 65 per cent. The synthesis of Farmer was also tolerable, but the overall yield was not too good. Therefore, although the cyclic 52 and 50 were not obtained from our method, the new synthesis of 47a proved to be good fortune indeed! The structure of the phosphite

polymer or oligomer was not investigated, but structure 53 is suggested as being a reasonable formulation. Figure 3 pictures the conversion of the cis-diol (51) into Carré's acid.

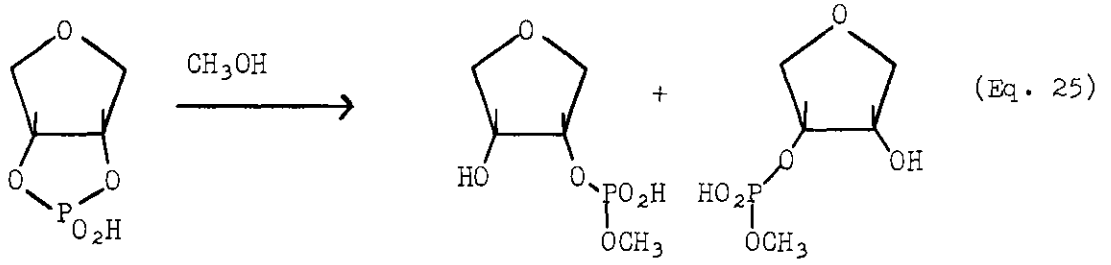


51 53 47a

Figure 13. The Conversion of the cis-Diol (51) into 47a.

Though a study of the triester (50) should be of interest in itself, this compound was desired primarily as an intermediate in the

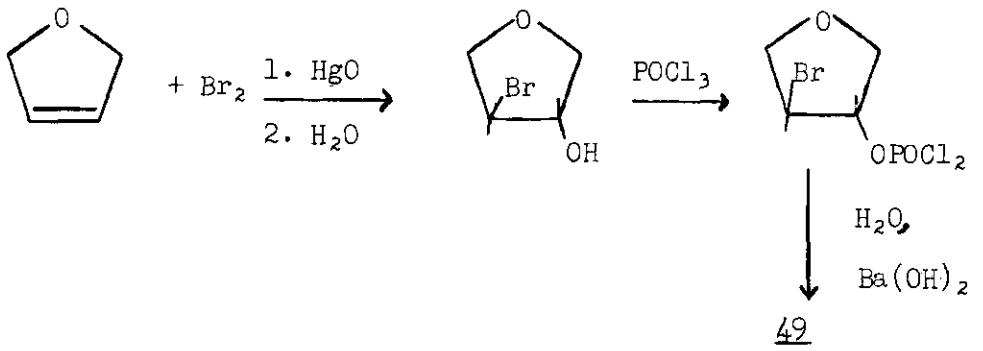
synthesis of 46. Since 50 was not easily obtainable, attention was turned toward the conversion of 47a into 46. Cox (32,34) observed that ethylene phosphate was hydrolyzed very rapidly in acid solution. My preliminary studies indicated that Carré's acid was also hydrolyzed rapidly in aqueous acid. It was reasonable, therefore, that the methanolysis (dry methanol) of Carré's acid would take place rapidly and would yield the desired diester (46b) (Eq. 25). This expectation was indeed realized. After neutralization of the reaction mixture in situ, excellent yields of 46b and 46c were obtained. No contamination by 47b was observed in the n.m.r. and IR spectra. A similar attempt to prepare the analogous phenyl ester (phenol as solvent) failed, presumably due to the decreased acidity of 47a in phenol and the smaller nucleophilicity of phenol relative to methanol. No preparative attempts at opening the five-cyclic phosphate ring of 47 in methanolic methoxide or phenoxide were made.



(Eq. 25)

47a 46a 46a

The bromohydrin phosphate (48) was easily prepared by the method of Cox (32,77). The details of the synthesis of the bromohydrin phosphate (49) will be presented else where, but the reactions used by Farmer are given below.

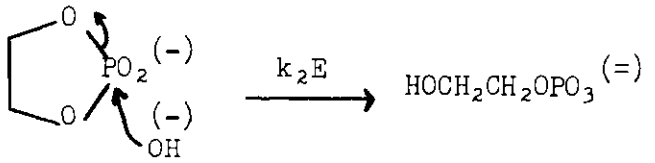


49

Kinetics

Ethylene Phosphate

Since the alkaline hydrolysis of ethylene phosphate (Eq. 26) is an overall second-order process (77), the rate dependence on temperature has been measured at 0.283 M hydroxide ion concentration only. The



(Eq. 26)

12 54

pseudo first-order and true second-order rate constants are given in Table 5. Duplicate runs have been made to determine each rate constant. All rate constants are estimated to be accurate within ± 3 per cent. An Eyring plot (109) of these data is reproduced in Figure 14. The pseudo first-order rate constant for the hydrolysis of cyclohexylammonium pinacol phosphate (Eq. 27) at 50° in 0.283 M base at an ionic strength

Table 5. Pseudo First-Order and True Second-Order Rate Constants for the Hydrolysis of Cyclohexylammonium Ethylene Phosphate in 0.283 M Hydroxide Ion Solution.

T°c	μ	$10^4 k_{obs}$ (sec.)$^{-1}$	$10^4 k_{2E}$ (m/l)$^{-1}$(sec.)$^{-1}$
32.7	0.30	1.85	6.52
39.7	0.30	3.00	10.6
49.7	0.30	6.09	21.5

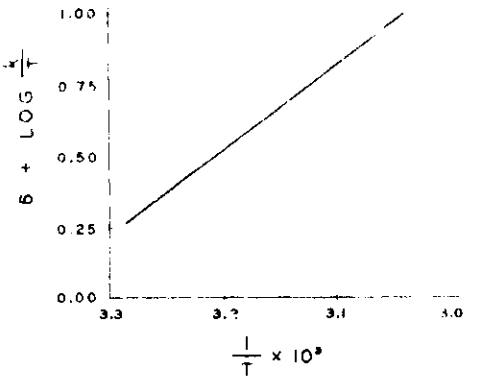
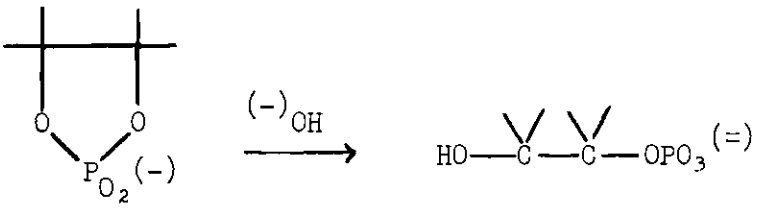


Figure 14. An Eyring Plot of the Data Given in Table 5.

of 0.30 is about 6 x 10^{-6} $(sec.)^{-1}$. No other hydrolytic rate data have been collected for this interesting five-cyclic diester.



(Eq. 27)

The pseudo first-order rate constants for the acid-catalyzed hydrolysis of ethylene phosphate at 0° and ionic strength 0.20 are presented in Table 6. As expected, of course, the observed rate constant

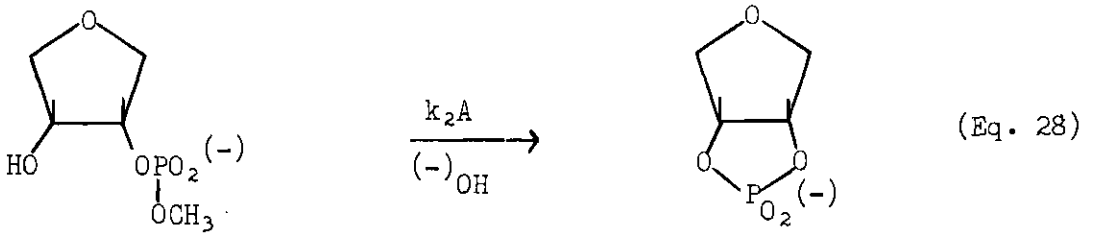
Table 6. Pseudo First-Order Rate Constants for the Acid-Catalyzed Hydrolysis of Barium Ethylene Phosphate.

T, °C	$[HClO_4]$ M	μ	$10^5 k$ $(sec.)^{-1}$
30	0.00487	0.20	0.512
30	0.0100	0.20	2.33
30	0.0146	0.20	4.91
30	0.100	0.20	214.
30	0.150	0.20	361.
30	0.00485	0.013	0.956
0	0.0395	0.20	1.97
0	0.0452	0.20	2.60
0	0.0600	0.20	4.70
0	0.0817	0.20	7.95
0	0.0995	0.20	11.6
0	0.124	0.20	17.2
0	0.160	0.20	26.8
0	0.184	0.20	35.3

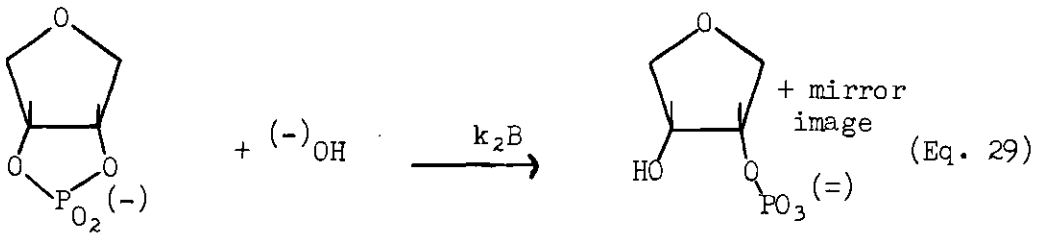
is a sensitive function of the pH of the solution. The data of Cox (32, 34) at 30° are also included in Table 6. Since barium ion does not act as a hydrolytic catalyst toward ethylene phosphate in acid solution (32), barium ethylene phosphate has been used. The pseudo first-order constant for the hydrolysis of the five-cyclic 47b is $8.87 \times 10^{-4}(\text{sec.})^{-1}$ at 0° in 0.292 M acid at an ionic strength of 0.30. A detailed analysis of the kinetics of hydrolysis of this ester in acid, however, will have to await further study.

The Tetrahydrofuranyl Esters (46b) and (47b)

Guida (55), in some preliminary studies of the alkaline hydrolysis of 46b and 47b, observed that the β-hydroxy ester (46b) cyclized with the loss of the elements of methanol at a rate comparable to the rate of saponification of the five-cyclic 47b (Eqs. 28 and 29). Hence, though a titration technique could be used to follow the rates of transesterification of 46b, it became desirable to employ a better analytical method.

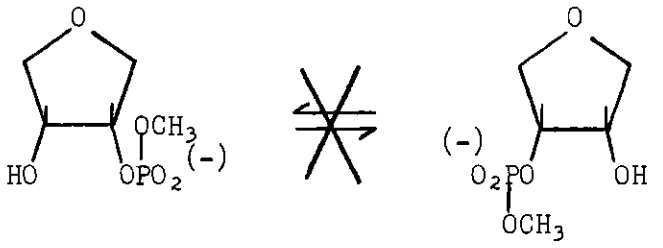


46b 47b (Eq. 28)



47b (Eq. 29)

The β-hydroxy ester (46b) was, therefore, resolved into its two optical isomers via the quinine salt (46c) (see experimental section). Since 47b is a symmetric molecule, the rate of racemization of 46b is then identical to the rate of transesterification of 46b to 47b. In view of the observation that other β-hydroxy esters, the ribonucleic acids in particular (19), do not undergo phosphoryl migration in base (22), a mechanism whereby racemization is brought about by phosphoryl migration without cyclization to 47b (Eq. 30) can be rejected. This argument is further supported by the observation that the transesterification rates estimated by a titration technique (55) are in agreement with those carefully measured polarimetrically.



(Eq. 30)

It is quite interesting that not only the magnitude, but also the sign of the specific rotation of the resolved 46b is a sensitive function of the solvent. Hence, the isomer showing $[\alpha]^{25}_{546} = +11$ in ethanol has $[\alpha]^{40}_{546} = -3.0$ in water, and vice versa. No effort was made either to determine the absolute configuration of each optical isomer or to study the temperature dependence of the specific rotations.

The pseudo first-order rate constants (k_{obs}) as a function of the base concentration only for the cyclization of 46b (Eq. 28) and the saponification of 47b (Eq. 29) are given in Tables 7 and 8, respectively.

The calculated true second-order rate constants (k_{2A} or k_{2B}), assuming a process which is first order in base, are also given in these two tables. The constancy of both calculated second-order rate constants (k_{2A} and k_{2B}) Clearly shows that both the transesterification ring closure (Eq. 28) and the hydrolysis reaction (Eq. 29) are overall second-order processes, first order in ester and first order in hydroxide ion (Eq. 31). It must

$$V = k_2 [E] [^{(-)}OH] \qquad \text{(Eq. 31)}$$

be noted carefully that both the internal transesterification reaction and the saponification reaction proceed at almost identical rates under a given set of conditions.

Table 7. Pseudo First-Order and True Second-Order Rate Constants as a Function of Base Concentration for the Base-Catalyzed Transesterification of 46b at 49.7° and Ionic Strength 0.30.

[NaOH] M	$10^4 k_{obs}(sec.)^{-1}$	$10^3 k_{2A}(m/l)^{-1}(sec.)^{-1}$
0.101	3.63	3.60
0.200	7.00	3.50
0.281	10.2	3.62

k_{2A} average = $3.57 \times 10^{-3}(m/l)^{-1}(sec.)^{-1}$

Table 8. Pseudo First-Order and True Second-Order Rate Constants as a Function of Base Concentration for the Base-Catalyzed Hydrolysis of 47b at 49.7° and Ionic Strength 0.30.

[NaOH] M	$10^4 k_{obs}(sec.)^{-1}$	$10^3 k_{2B}(m/l)^{-1}(sec.)^{-1}$
0.101	3.32	3.29
0.200	6.69	3.35
0.281	9.70	3.45

k_{2B} average = $3.36 \times 10^{-3}(m/l)^{-1}(sec.)^{-1}$

In Tables 9 and 10 are listed the results of detailed kinetic studies of the base-catalyzed cyclization of 46b (Eq. 28) and the hydrolysis of 47b (Eq. 29), respectively. Each true second-order rate constant has been calculated from the appropriate k_{obs} and hydroxide ion concentration parameters. The k_{obs} values used in the calculations of each second-order constant are averaged values obtained from two or more separate kinetic runs. All but one of the rate constants for the cyclization of 46b have been measured on the levo (water) isomer. The value reported in number 6, Table 9, is the rate constant for the cyclization of the dextro (water) isomer. All rate constants reported in these two tables should be accurate within ± 3 per cent.

The results listed in Table 9 (numbers 3 and 4) and Table 10 (numbers 2 and 5) clearly establish that both reactions are subject to a positive salt effect, as expected, as well as catalysis by barium ion (numbers 4 and 5 in Table 9, and numbers 5, 6, and 7 in Table 10). It

Table 9. Rate Data for the Base-Catalyzed Cyclization of 46b.

No.	T°c	[NaOH] M	μ	$[Ba^{(+2)}]$ M	$10^3 k_{2A}(m/l)^{-1}(sec.)^{-1}$
1	60.2	0.101	0.30	0	7.18
2	49.7(1)	- - -	0.30	0	3.57(1)
3	39.7	0.101	0.30	0	1.79
4	39.7	0.101	0.50	0	2.16
5	39.7	0.101	0.50	0.065	5.12
6	39.7(2)	0.101	0.30	0	1.81
7	49.7(3)	0.285	0.30	0	3.38

(1) The averaged value from Table 7; (2) obtained using the dextro (water) isomer; (3) $NaOD/D_2O$.

may be significant that the barium ion catalysis observed in the saponification of 47b is about twice that observed in the cyclization of 46b. Reassuring indeed is the observation that the rate constants for the racemization of both optical isomers of 46b are identical (numbers 3 and 6 in Table 9). The value 1.06 for the solvent deuterium kinetic isotope effect is readily extracted from rate constants 2 and 7 in Table 9. The significance of this number will be dealt with further in Chapter IV of this thesis. The activation parameters obtaining for the reactions portrayed by Equations 28 and 29 have been extracted from the appropriate data in Table 9 (numbers 1, 2, and 3) and in Table 10 (numbers 1, 2, 3, and 4) by means of the Eyring equation (109); Figures 15 and 16 are reproductions of the respective Eyring plots.

Table 10. Rate Data for the Base-Catalyzed Hydrolysis of 47b.

No.	T°c	[NaOH] M	μ	$[Ba^{(+2)}]$ M	$10^3 k_{2B}(m/l)^{-1}(sec.)^{-1}$
1	60.4	0.0841	0.30	0	6.67
2	49.7 (1)	------	0.30	0	3.36 (1)
3	39.7	0.0841	0.30	0	1.72
4	32.7	0.0841	0.30	0	1.09
5	49.7	0.101	0.50	0	4.02
6	49.7	0.101	0.50	0.065	18.8
7	39.7	0.101	0.50	0.065	8.90

(1) The averaged value from Table 8.

The enzyme ribonuclease appears neither to catalyze the internal transesterification of 46b nor the hydrolysis of 47b. That the lower acidity of D_2O relative to H_2O is responsible for our failure to observe enzymatic catalysis can be tentatively rejected by a mental extrapolation of the observations of Barker and coworkers (8) who have found that ribonuclease catalyzes the solvolysis of uridine-2'-3' cyclic phosphate in solutions of aqueous primary, but not secondary, alcohols. It must be emphasized that competitive inhibition studies have not been performed, and therefore, the question of a possible enzyme-substrate complex must remain moot.

The Bromohydrin Phosphates (48 and 49)

The cyclizations of the barium salts of the two bromohydrin phosphates (48 and 49) are illustrated by equations 32 and 33, respectively. The true first-order rate constants for each of these two processes

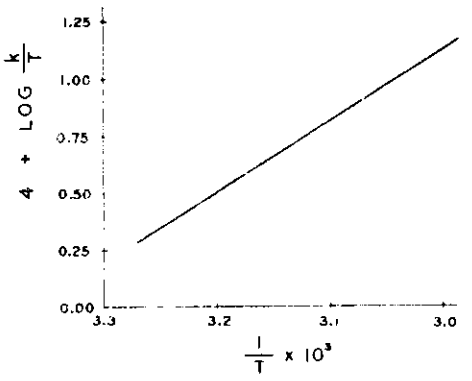


Figure 15. The Eyring Plot of the Appropriate Data in Table 9.

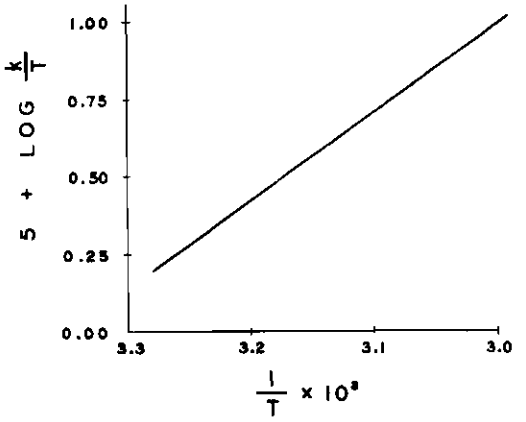
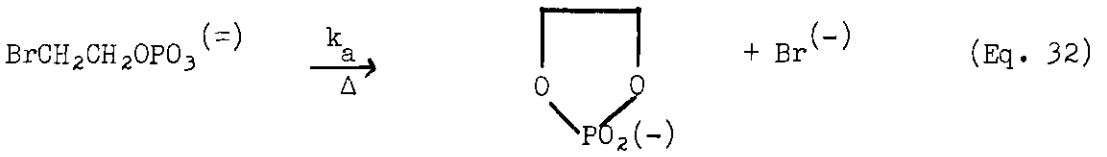


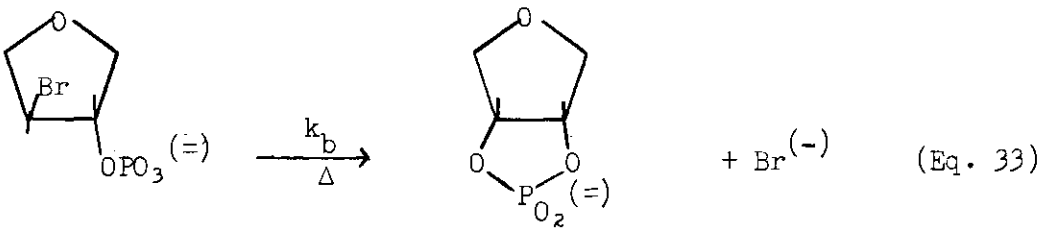
Figure 16. The Eyring Plot of the Appropriate Data in Table 10.

(Eq. 32 and Eq. 33), as measured in a 0.033 M borax buffer (pH 9) are



(Eq. 32)

48 12



(Eq. 33)

49 47

listed in Tables 11 and 12, respectively. The accuracy of these data is estimated to be ± 3 per cent. As previously stated, these two reactions have been used to synthesize the five-cyclic diesters 12 and 47 (32,48).

Table 11. The First-Order Rate Constants for the Cyclization of the Barium Salt of 48 in a 0.033 M Borax Buffer.

T°c	μ	[$NaClO_4$] M	$10^4 k_a (sec.)^{-1}$
39.7	0.20	0.05	1.28
49.7	0.20	0.05	4.47
57.8	0.20	0.05	11.5
61.8	0.20	0.05	18.5

Table 12. The First-Order Rate Constants for the Cyclization of The Barium Salt of 49 in a 0.033 M Borax Buffer.

T°c	μ	[$NaClO_4$] M	$10^4 k_b (sec.)^{-1}$
65.9	0.20	0.05	0.363
70.9	0.20	0.05	0.682
75.9	0.20	0.05	1.26
75.9	0.60	0.45	1.21
61.8 (extrap.)	(0.20)	(0.05)	0.219

Both sets of data produce good straight lines when subjected to an Eyring plot (109). Figure 17 is a reproduction of the Eyring plot of the data given in Table 11. As expected, the cyclization of 49 (Eq. 33) is subject to a very small negative salt effect. This is, presumably, also true for the cyclization of 48 (Eq. 32). That 48 cyclizes some 90 times faster than 49 at 61.8° is evident from the rate constants in Tables 11 and 12.

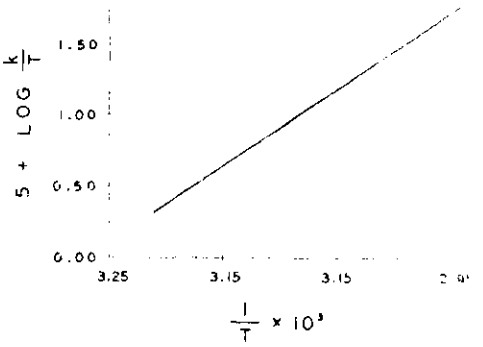


Figure 17. An Eyring Plot of the Data in Table 11.

CHAPTER IV

DISCUSSION

Cox (32,34) has observed that the rate equation governing the hydrolysis of ethylene phosphate (in acid at 30°) contains only the term for the hydrolysis of the conjugate acid of ethylene phosphoric acid (Eq. 13). A careful examination of our data (Table 6) reveals that

$$V = k_2 (HA)(H^{(+)}) \qquad \text{(Eq. 13)}$$

Where HA is ethylene phosphoric acid.

this behavior also obtains for this hydrolysis reaction at zero degrees. The two plots of $-\log k_{obs}$ *vs.* pH for the two sets of data presented in Table 6 give good straight lines of slopes 2.1 and 2.0 at 30 and zero degrees, respectively, in regions of low acidity (*i.e.*, $(H^{(+)}) \leq 0.1$ M). In regions of higher acidity, curved lines, the slopes of which deviate from a value of two toward a value of one, are obtained.* This very interesting behavior is predicted exactly from equations 36, 36a, and 36b, which are readily derivable from equations 34 and 35.

Since the ionization constant of ethylene phosphoric acid (K_i) appears in equation 36, one is forced to evaluate this constant as a function of temperature if the true second-order rate constant (k_2) is

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* Unfortunately the rate becomes too rapid to measure before this behavior is fully developed.

to be determined. Bunton and coworkers (25) have measured directly the ionization constant of dimethyl phosphoric acid as a function of temperature and have extrapolated these values to 100° where the hydrolysis

$$V = k_{obs} (E) \qquad \text{(Eq. 34)}$$

Where (E) is the stoichiometric ester concentration

$$K_i = (H^{(+)})(A^{(-)})/_{(HA)}) \qquad \text{(Eq. 35)}$$

Where $(E) = (HA) + (A^{(-)})$, then

$$k_{obs} = k_2 (H^{(+)})^2/_{K_i} + (H^{(+)}), \text{ and} \qquad \text{(Eq. 36)}$$

$$k_{obs} = \frac{k_2}{K_i} (H^{(+)})^2 \text{ if } K_i \gg (H^{(+)}) \text{ or} \qquad \text{(Eq. 36a)}$$

$$k_{obs} = k_2 (H^{(+)}) \text{ if } (H^{(+)}) \gg K_i \qquad \text{(Eq. 36b)}$$

rates of this ester have been measured. But, as Nature would have it, the ethylene phosphate ring is much too labile to remain intact in the presence of aqueous acid, and, therefore, we have been forced to estimate the K_i values from the kinetic data given in Table 6 and equation 36. With the aid of a Burroughs B-220 digital computer, itterative calculations of k_2 as a function of K_i have been performed by substituting the measured k_{obs} and $(H^{(+)})$ parameters and assumed K_i values into equation 36. For each set of data in Table 6, the value of K_i for which a constant, acidity-independent value of k_2 is obtained has been taken as the ionization constant of ethylene phosphoric acid under the appropriate conditions

of temperature and ionic strength. Confidence in this method is strengthened by our observation that each of the two plots (for zero and 30°) of the standard deviations in the calculated k_2 values *vs.* the assumed K_i values is a concave downward curve possessing one distinct minimum corresponding to our chosen values of k_2 and K_i.

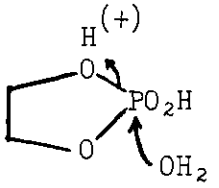
The k_2 and K_i values so obtained are presented in Table 13. Though our value of K_i at 30° differs somewhat from the value 0.1 first estimated graphically by Cox (32,34), the agreement between the two numbers is really quite good, considering the errors inherent in the method. We also feel that the newer K_i value is probably more accurate than the old, and, at any rate, the two K_i parameters reported in Table 13 have been ob-

Table 13. The Values of K_i and k_2 Calculated from the Data in Table 6 and Equation 36.

T°c	μ	K_i	$10^2 k_2$ $(m/l)^{-1}(sec.)^{-1}$
0.0	0.20	0.48	0.673
30.	0.20	0.30	7.33

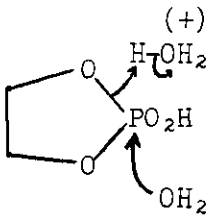
tained by the same method of calculation. As expected, the ionization constant is indeed a temperature dependent function, although not nearly so sensitive a one as is the rate constant. To be noted in passing is Harned and Embree's demonstration that the ionization constants of phosphoric acids reach maximum values in the temperature range of zero to 100° (60). The effect of ionic strength on K_i has not been studied.

A brief statement concerning the actual mechanism of the acid-catalyzed hydrolysis of ethylene phosphate is in order. The rate equation (Eq. 13) taken with the position of bond fission (exclusively P-O) can be interpreted as evidence for a nucleophilic attack of a water molecule on the ethylene phosphoric conjugate acid species (Eq. 37). Indeed, this is a very good interpretation of the data at hand. Nevertheless, there is another mechanism which may be operating, for or



⟶ intermediate or products (Eq. 37)

against which there is presently available no experimental evidence. Simply stated, the acid-catalyzed hydrolysis of ethylene phosphoric acid may be subject to general acid catalysis (Eq. 38). The hydrolytic catalysis in aqueous base of some heavy metal ions (see Chapter III)



⟶ intermediate or products (Eq. 38)

has been attributed to a "general-acid" catalysis process in which the metal ion (e.g. barium ion) is really acting as a general acid (77). Some enzymes, especially the hydrolytic enzymes (e.g. chymotrypsin and ribonuclease) may, and probably do, act as both general acids and general or nucleophilic bases. The problem of proving or disproving

general acid catalysis in the ethylene phosphate hydrolysis is not a simple one and will probably remain unsolved, especially since ethylene phosphate is, in some respects, a rather poor model of the intermediate, cyclic nucleotides. Yet, the possibility of a general acid catalysis mechanism is quite important and must be sought after in a study of better and more sophisticated nucleic acid models.

It must be recalled that the rate equation (Eq. 5) for the acidic hydrolysis of dimethyl phosphate contains two terms, one for the neutral species (≈ 25 per cent P-O cleavage) and one for the conjugate acid (0-11 per cent P-O cleavage (25,56). In Chapter I it has been suggested that the rate equation (Eq. 13) for the acid-catalyzed hydrolysis of ethylene phosphate (100 per cent P-O cleavage) constitutes evidence that the phosphorus atom in a five-cyclic phosphate possesses an enhanced positive charge relative to one in an acyclic ester. If the central phosphorus atom bears an enhanced positive charge, then, of course, the surrounding oxygen atoms must bear an enhanced negative charge and hence be more susceptible to protonation. Furthermore, if the five-cyclic phosphorus atoms do bear an enhanced charge, one would expect this electronic characteristic to be evident in the energetics, in particular the activation energetics, of the reactions at phosphorus of cyclic vs. acyclic esters. With this consideration in mind, the activation parameters for the hydrolysis of ethylene phosphate in both acid and base and of Carré's salt (47b) in base have been measured.

The appropriate activation parameters are presented in Table 14. The activation free energy ($\Delta G^{\ddagger}$), enthalpy ($\Delta H^{\ddagger}$), and entropy ($\Delta S^{\ddagger}$) for the alkaline hydrolysis of ethylene phosphate (12) and of Carré's salt

(47b) have been calculated from the rate data in Tables 5 and 10 (Figures 14 and 16), respectively; the two rate constants presented in Table 13 have been used to obtain the $\Delta H^{\ddagger}$ parameter for the acid-catalyzed hydrolysis of ethylene phosphoric acid. The activation parameters for the base-catalyzed hydrolysis of dimethyl phosphate (32) and the acid- and base-catalyzed hydrolyses of the five-cyclic phosphonates (46) have been calculated from published rate data.

In as much as the various heat capacities of activation ($\Delta Cp^{\ddagger}$) have been ignored, an inherent error is present in all the results listed in Table 14. This error should be small, however, especially since comparisons are to be made among structurally similar compounds. A much larger error in the activation parameters pertaining to the hydrolyses of dimethyl phosphate and ethyl ethylphosphonate emerges as a direct corollary to the error in measuring accurately the rate constants for the P-O only and C-O only cleavage reactions (46,56). The experimentally determined rate constants for the hydrolysis of the acyclic phosphate ($\approx$ 10 per cent P-O cleavage) and phosphonate ($\approx$ 50 per cent P-O cleavage) have, accordingly, been corrected by multiplication by factors of ten and two, respectively, to yield values representative of the P-O cleavage processes only. The $\Delta G^{\ddagger}$ values calculated from the corrected rate constants should be fairly good. The $\Delta H^{\ddagger}$ parameters, on the other hand, are inseparable composites of the appropriate parameters for both P-O and C-O cleavage processes and must, therefore, be regarded as only crude approximations to the separate values of interest.

Since only P-O cleavage processes have been observed in the hydrolyses of the five-cyclic esters (46,55,56), the activation parameters

obtaining for these reactions are not clouded by contributions from any C-O fission pathways. Nevertheless, any long extrapolation may be subject to large error; hence, the appropriate $\Delta G^{\ddagger}$ parameters for the hydrolyses of cyclic and acyclic esters have been compared at both 25 and 125°. Since the appropriate $\Delta\Delta G^{\ddagger}$ values are rather large, the extrapolation errors have little effect upon conclusions drawn therefrom. In view of the uncertainties in determining the ionization constants of the cyclic phosphoric and phosphonic acids and the lack of temperature-dependent rate data on the hydrolysis of dimethyl phosphoric acid, no attempt has been made to compare the acid-catalyzed hydrolysis activation parameters of the cyclic vis á vis the acyclic esters. Possible ionic strength effects, ignored in the calculations of the parameters listed in Table 14, will be dealt with later in the discussion of the transesterification reaction of 46b.

Table 14. The Activation Parameters for the Hydrolyses of Several Cyclic and Acyclic Phosphates and Phosphonates.

Ester	Catalyst	$\Delta G^{\ddagger}$(T°c) (kcal/mole)	$\Delta H^{\ddagger}$ (kcal/mole)	$\Delta S^{\ddagger}$ (e.u.)
Ethylene Phosphate	Base	22.3(25°) 25.2(125°)	13.1	-30.4
Ethylene Phosphate	Acid	---------	12.5	----
Carré's Salt (47b)	Base	21.9(25°)	12.8	-30.3
Dimethyl Phosphate	Base	33.(25°) 35.(125°)	26.	----
Propyl Phostonate	Base	23.9(25°) 26.6(125°)	15.4	-28.2
Propyl Phostonate	Acid	---------	14.	----
Ethyl Ethylphosphonate	Base	32.7(25°) 35.4(125°)	23.5	----

The most striking aspect of the data in Table 14 lies in the low values of the activation enthalpies for the hydrolyses of the five-cyclic phosphates. At first glance the activation enthalpies of the two cyclic phosphates appear to be about 13 kcal/mole less than that estimated for the acyclic ester. However, since the acyclic ester hydrolyzes in base with only about ten per cent P-O cleavage, these $\Delta\Delta H^{\ddagger}$ values are comparisons of P-O vs. C-O cleavage reactions. The transition state for the P-O cleavage hydrolysis (base) of dimethyl phosphate is surely much more polar than that for the C-O cleavage hydrolysis. Hence, by analogy with data for other systems (1,52) both the activation enthalpy and the activation entropy for a strictly P-O cleavage process may be less than those values actually determined experimentally. Since an estimation of the $\Delta H^{\ddagger}$ value for the P-O fission process from Cox's data (32) would be mere speculation at best, one is forced to turn to a consideration of the fairly accurate $\Delta G^{\ddagger}$ values for the P-O fission hydrolyses of both the cyclic and acyclic esters.

It is assumed in this discussion that all the driving forces for the hydrolysis of the cyclic phosphates are satisfied in the hydrolytic transition states. An inspection of the activation energies listed in Table 14 shows clearly that the cyclic-acyclic $\Delta\Delta G^{\ddagger}$ value for the base-catalyzed hydrolyses is 10-10.5 kcal/mole. (This $\Delta\Delta G^{\ddagger}$ value is the approximate average of the values taken at 25 and 125°.) If one assumes that the activation enthalpy of the cyclic ester is lower than that of the acyclic ester by about 5.5 kcal/mole, the "strain" energy measured for methyl ethylene phosphate, one then calculates an activation entropy of $\approx$ -45 e.u. for the P-O fission, base-catalyzed hydrolysis

of dimethyl phosphate. This number is ridiculously small for the reaction in aqueous solution of two negative ions with each other* and, hence, this energy model may be rejected. The opposite extreme may be taken, of course, by assuming that the five-cyclic ester phosphorus atoms do not bear any enhanced positive character and, therefore, that the cyclic-acyclic $\Delta\Delta S^{\neq}$ value for the strictly P-O cleavage reactions should be roughly zero. This model then requires that the "strain" in the five-cyclic diester (relative to the acyclic diester) be about 10 kcal/mole, nearly twice that measured in the five-cyclic triester. I feel that this is a most unlikely state of affairs indeed and, therefore, that this energy scheme may also be rejected.

The best interpretation of the data is obtained by assuming that the activation enthalpy of the cyclic diester has been lowered from that of the acyclic analogue by a term corresponding to the measured triester strain, 5.5 kcal/mole, plus an additional term attributable to either the additional strain in the cyclic diester (suggested in Chapter I) or the enthalpic result of an enhanced positive charge on the phosphorus atom, or both. For the purpose of this argument I have assumed that this additional term is worth about 3 kcal/mole. The base-catalyzed hydrolytic $\Delta H^{\neq}$ and $\Delta S^{\neq}$ for dimethyl phosphate would then become about 21.5 kcal/mole and -36 e.u., respectively. A $\Delta S^{\neq}$ value of about -36 e.u. is not at all an unreasonable value for this number. If there is really

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* The smallest $\Delta S^{\neq}$ value listed by Frost and Pearson (50) is -41 e.u., the value obtaining for the reaction in aqueous solution of two $S_2O_4^{(=)}$ ions with each other.

more charge separation in the cyclic than in the acyclic esters, the ground states of the cyclic compounds must be more solvated than the ground state of the acyclic analogue. However, if one returns to the oft repeated assumption that the driving forces for the hydrolysis of the cyclic esters are completely satisfied in the transition states (i.e., that the transition states for the base-catalyzed hydrolyses of the cyclic and acyclic esters are about the same electronically), one must come to the unavoidable conclusion that less additional solvation is required to reach the hydrolytic transition state of the cyclic esters than is required to reach the electronically similar transition state for the acyclic analogue. Hence, less entropy would be lost in reaching the five-cyclic hydrolytic transition state than would be lost in reaching the corresponding acyclic hydrolytic transition state. The net result of an enhanced positive charge on phosphorus could, therefore, be manifest in both an enthalpy and an entropy term.

At this point it should be emphasized that this discussion is but one man's interpretation of the experimental evidence. Nevertheless, I feel that the data strongly supports the theory that the strain energy of 5.5 kcal/mole, long associated with the strain in the ethylene phosphate ring, cannot be the only enthalpy term influencing the reactivity of this ester (56,71). It is therefore suggested that an additional enthalpy term, above chosen arbitrarily at about 3 kcal/mole, is influencing the reactivities of the five-cyclic esters. This term, of course, may be associated with additional strain in the cyclic diester as well as an enhanced positive charge on the phosphorus atom. It appears unreasonable that this additional strain should amount to 4.5-5.0 kcal/mole;

therefore it is also suggested that an entropy term, associated with the enhanced positive charge on phosphorus, is partly responsible for the rate acceleration observed.

Naturally, it is quite simple to propose and discuss these corollaries; it is not so simple to obtain experimental evidence in their support. It would appear that an acyclic diester, suitably "tricked" to react exclusively by a P-O cleavage pathway, would not serve as a very good comparison model. The arguments given above, however, also apply to the triester series where only P-O cleavages are observed under alkaline conditions. Thus, arguments concerning reaction intermediates notwithstanding, the activation enthalpy and entropy for the alkaline hydrolysis of methyl ethylene phosphate, for which a reasonably accurate "strain" energy has been measured, should shed some light on the postulates pertaining to additional enthalpy and entropy terms.

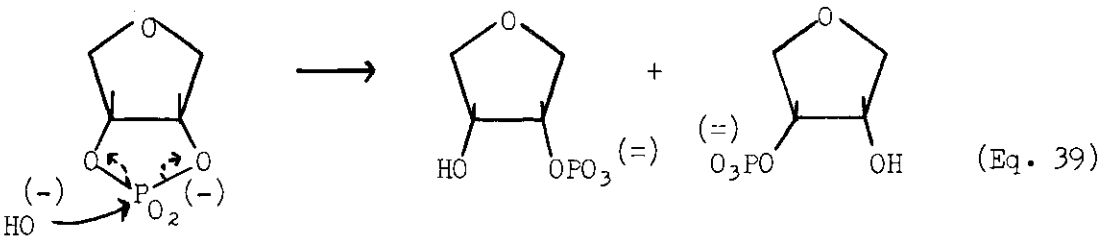
The absence of a reasonable strain estimate for the cyclic phosphonate esters renders impossible even a superficial analysis of the $\Delta\Delta G^{\neq}$ value in this system. That the $\Delta\Delta G^{\neq}$ value is smaller in the phosphonate than in the phosphate system is, of course, only a restatement of Eberhard and Westheimer's (46) kinetic results. Nevertheless, it is interesting that both the activation enthalpy and entropy obtaining for the base-catalyzed hydrolyses are larger in the cyclic phosphonate than in the cyclic phosphate system. This observation is analogous to the activation enthalpy-entropy leveling effect found in other systems by Aksnes (1) and Ginjaar and Blasse-Vel (52) and may be interpreted as evidence that the five-cyclic phosphates are more polar than the corresponding phosphonates. This interpretation must be accepted with

caution, however, since one can easily argue that the cyclic phosphonate is not so strained as the cyclic phosphates. Furthermore, the difference in the two $\Delta S^{\neq}$ values is really rather small and may only reflect the experimental error in their measurement.

The relatively low magnitudes of the activation enthalpies for both the acid- and base-catalyzed hydrolyses of ethylene phosphate are potentially significant with regard to the energetics of an enzyme-catalyzed hydrolysis. As already noted in this thesis, an enzyme such as ribonuclease is neither a strong proton donor nor an exceptionally good nucleophile, and, therefore, most probably functions by means of a complex bifunctional catalysis (i.e., general acid-general or nucleophilic base) mechanism. In so far as the activation enthalpies are concerned, the data in Table 14 indicate that the behavior of one ethylene phosphoric acid molecule toward a water molecule and one additional proton is much the same as the behavior of one ethylene phosphate monoanion toward a hydroxide ion. This similarity in reactivity points out that, with regard to the low heats of activation, the superficially simple ethylene phosphate is really a fairly respectable model of the naturally-occurring intermediate cyclic nucleotides, the hydrolyses of which are catalyzed by ribonuclease (28).

Another very important comparison is obtained from an examination of the rate data (Tables 5 and 10) for the hydrolyses of ethylene phosphate and Carré's salt (47b). As expected, the product analysis (55) and kinetic data demonstrate that the base-catalyzed hydrolysis of Carré's salt and ethylene phosphate proceed by the same mechanistic pathway: namely, the attack of a hydroxide ion on phosphorus concurrent

with the ring-opening expulsion of an alkoxide anion (Eq. 39). Furthermore, the structurally much more complex Carré's salt is attacked by



47b (Eq. 39)

hydroxide ion only about 1.6 times faster than is ethylene phosphate while the activation enthalpies and entropies for the base-catalyzed hydrolyses of the two esters are actually well within experimental error of each other (Table 14)! The great importance of this result can be grasped through a consideration of the structural relationships among ethylene phosphate, Carré's salt, and the intermediate, cyclic nucleotides. Ethylene phosphate, of course, is the simplest five-cyclic diester. Carré's salt possesses not only the five-membered phosphate ring but also the basic skeleton of the ribose sugar ring, the backbone of the ribonucleic acids. Structurally, the intermediate cyclic nucleotides may be regarded as a Carré's ester moiety which has been substituted with the 5'-hydroxymethyl and 2'-heterocyclic base groups. A priori, one might have expected the fused 3,4-tetrahydrofuran ring to have altered the reactivity at phosphorus of Carré's salt relative to ethylene phosphate. The presence of this fused ring, however, has almost no effect upon the reactivity of this five-membered phosphate! The high reactivity of Carré's salt and the intermediate cyclic nucleotides must, therefore, be tied to a large extent directly to the rate-accelerating

factors incorporated into the five-cyclic phosphate grouping. Although the thermodynamic strain present in the phosphate ring of Carré's salt has not been estimated, this strain value is probably about the same as that estimated for the ethylene phosphate ring (see above discussion). This tenet is certainly supported by the activation data in Table 14.

The respective leaving groups in the alkaline hydrolysis of Carré's salt and ethylene phosphate would appear to be somewhat different electronically. The inductive effect of the tetrahydrofuran ring ether oxygen atom in Carré's salt, however, apparently eliminates this formal electronic difference. Support for this hypothesis may be found in the data of Ballinger and Long (7) who have measured the acidities in water of several variously substituted primary alcohols. In contrast, the inductive effect of the 2'-heterocyclic base groups may enhance the reactivity of the intermediate cyclic nucleotides relative to Carré's salt and ethylene phosphate. In addition, the reactivities of the cyclic nucleotides may be further affected by the conformation of the ribose sugar ring. The steric interactions between the 5'-hydroxymethyl group and the 2'-base group would surely alter the conformation of the cyclic nucleotides relative to the conformation of Carré's salt. Although these expected reactivity effects must be evaluated if the chemistry of the nucleic acids is to be understood, they should certainly be small compared to the enormous effect imparted by the five-membered phosphate moiety.

The most exciting aspect of our research has come from our comparison of the rates of cyclization of 46b (Eq. 28) with the rates of hydrolysis of Carré's salt (47b) (Eq. 29). The depolymerization of the

ribonucleic acids, it must be recalled, is the faster step in the overall depolymerization and hydrolysis of these esters (Figure 1). In direct opposition to the behavior of these naturally occurring substrates is the behavior of Brown and coworker's models (17,18,19,22,23), where the internal transesterification of the β-hydroxy diesters (except the substituted phenyl diesters) is slower than the subsequent solvolysis of the five-membered phosphates (Eqs. 9 and 10). However, as the rate data in Tables 9 and 10 demonstrate so well, the order of reactivities observed by Brown's group is definitely not observed on our system; indeed, the two esters (46b and 47b) react at almost identical rates (k_{2A}/k_{2B} = 1.06 at 50°)! Thus, while the cis-fused tetrahydrofuran ring has almost no effect upon the saponification rate of the five-cyclic phosphate grouping structurally contained in Carré's salt, this same ring has a very large effect upon the intramolecular transesterification rate of the Carré's salt precursor (46b)!

The kinetic (Chapter III) and product analysis results (55) clearly delineate 47b as the immediate phosphorus-containing product derived from the base-catalyzed cyclization of 46b. Conceivably, there are open to 46b (and other β-hydroxy diesters) two plausible reaction pathways (Eqs. 40 and 41), both of which would obey the experimentally determined rate equation (Eq. 31).* The pathway illustrated by equation 40, the specific hydroxide ion-catalyzed pathway, demands the formation of an intermediate alkoxide anion in an equilibrium step. This very reactive alkoxide anion may then either revert to the starting ester or cyclize

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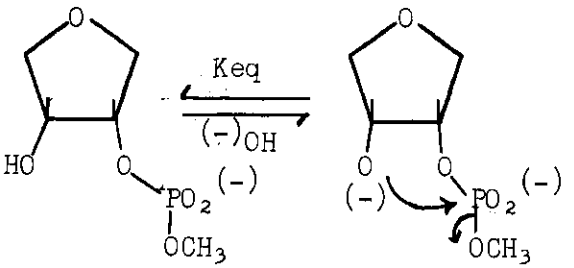
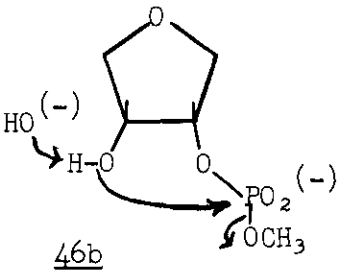
* Assuming the acid-base equilibrium (Eq. 40) to be rapidly established.



46b

$\xrightarrow{k_t}$ 47b (Eq. 40)



46b

$\xrightarrow{k'_{2A}}$ 47b (Eq. 41)

47b $\xrightarrow[k_{2B}]{(-)_{OH}}$ product

with the expulsion of a methoxide anion. The rate expression for this process is given by equation 42. Equation 41 represents a general base-

$$V = k_{2A}[E][OH^{(-)}] = K_{eq}k_t[E^{(-)}] \qquad \text{(Eq. 42)}$$

catalyzed pathway* in which no intermediate alkoxide anion is formed. Rather, the alcoholic proton is removed by the base concertedly with the attack of the nucleophile at phosphorus and the expulsion of the leaving group. Since the transition state of the general base mechanism would

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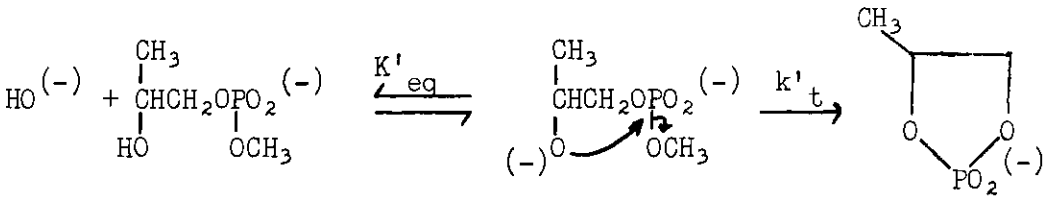
* The importance of a general base catalysis process has already been mentioned in this thesis.

contain both the starting ester and one hydroxide anion, the rate expression for this process is given by equation 31.

Unfortunately, 46b cyclizes too slowly in the pH range 9-11 to allow the search for general base catalysis by conventional means. Nevertheless, the magnitude of the solvent deuterium kinetic isotope effect should be indicative of mechanism. If the general base catalysis mechanism were operating, one would expect a k_H/k_D significantly greater than unity (31,43,64,69); if the specific hydroxide ion mechanism were operating, a $k_H/k_D \approx 1\text{-}2$ would be expected (12,64). Experimentally, a $k_H/k_D = 1.06$ has been observed. This single piece of evidence is certainly not enough to permit one to exclude rigorously the general base catalysis mechanism. On the other hand, it presents a fairly good argument in favor of the specific catalysis pathway, and the general base process will therefore be rejected at this time. Brown and Usher (22) also claim that the internal transesterifications of their simpler models proceed by a specific hydroxide ion mechanism analogous to that given by equation 40. Our failure to observe general base catalysis in the racemization of 46b certainly does not preclude that possibility in the cyclization of other esters with better leaving groups, for example, the related p-nitrophenyl ester, 55, in which the loss of a p-nitrophenoxide anion may compete favorably with the equilibrium formation of the intermediate alkoxide anion (see page 109). Brown and Usher (22) have observed that the p-nitrophenyl 2-hydroxypropyl phosphate cyclizes very fast in aqueous base. Though these two workers have not detected general base catalysis, they agree that their preliminary experiments have by no means excluded such a mechanism.

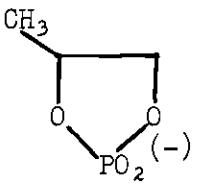
The equilibrium constant, K_{eq}, is not known and must be approximated from data published in the literature. Values given by Hine and Hine (65) for the acidity of water and other weak acids in 2-propanol may be manipulated to yield a $K_{eq} \approx 10^{-3}$ for the reaction in water of 2-propanol with hydroxide ion at 27°. From the data of Bruice and coworkers (24) on the hydrolysis rates of a series of acetate esters a $K_{eq} \approx 10^{-3} - 10^{-5}$ may be calculated for this same reaction at 25°. Ballinger and Long (7) have measured the ionization constants in water at 25° of a series of substituted, primary alcohols and have observed that their values in water parallel closely the values obtained by Hine and Hine in 2-propanol. Assuming that this analogy extends to secondary alcohols, a $K_{eq} \approx 10^{-3}$ may be calculated for the reaction of 2-butanol with aqueous hydroxide ion. Ballinger and Long have also observed that one β-methoxyl group lowers the pK_i of ethanol approximately one pK_i unit. It is therefore quite reasonable to assume the K_{eq} in equation 40 to be $\approx 10^{-2}$. Using $K_{eq} \approx 10^{-2}$ and the rate data in Table 9, a $k_t = k_{2A}/K_{eq} = 6.65 \times 10^{-4}/K_{eq} \approx 0.07$ sec.$^{-1}$ at 27° may be calculated. This k_t value represents the first-order rate constant for the cyclization of the alkoxide anion only and is to be compared with the $k_{2B} = 7.24 \times 10^{-4}$ (m/l)$^{-1}$ (sec.)$^{-1}$ at 27° (calculated from the data in Table 10), the rate constant for the saponification of Carré's salt. Hence, the alkoxide ion, once formed, cyclizes at least 10-100 times faster than 47b opens in one molar base. The true $k_t/k_{2B}^{(-)}(OH)$ ratio may be much larger.

The best comparison data come from the work of Brown and Usher (22) who have studied the base-catalyzed cyclization of methyl 2-hydroxypropyl phosphate, 56 (Eq. 43). Extrapolation of the two rate constants



56 57 (Eq. 43)

$$V = k_{2obs}[E][^{(-)}OH] = K'_{eq}k'_t\,(E^{[-)})$$



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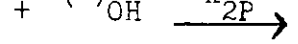

(Eq. 44)

reported at 60 and 80° to 27° yields a $k_{2obs} = 2.25 \times 10^{-6}(m/l)^{-1}(sec.)^{-1}$ at an ionic strength of one molar. Assuming* that $K'eq \approx Keq \approx 10^{-2}$, one obtains $k'_t \approx 2 \times 10^{-4}\ (sec.)^{-1}$. The rate constant k_{2P} is not precisely known but can be estimated from the report of Ukita, Nazasawa, and Irie (101) and the hydrolysis rate of ethylene phosphate. Since the Japanese workers have observed the barium salt of 1,2-propandiol cyclic phosphate to be hydrolyzed about four times slower than the sodium salt of ethylene phosphate, a $k_{2P} \approx 1 \times 10^{-4}\ (m/l)^{-1}\ (sec.)^{-1}$ (assuming a second-order process) at 27° can be calculated. Hence, the alkoxide anion of 56 cyclizes at only about the same rate as the 1,2-propandiol cyclic

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* In an attempt to evaluate the relative acidities of the hydroxyl groups in 46b and 56, I have equated in 46b the inductive, ionization-promoting effect of the β-ether linkage with the inductive (or field) ionization-retarding coulombic repulsion between the rigidly held phosphoryl and alkoxide anions. After making this assumption, it becomes quite reasonable to assume that $K_{eq} \approx K'_{eq}$.

phosphate opens in one molar base!

Admittedly, some of these estimated results, particularly those pertaining to Brown's model, are rather crude. Nevertheless, they clearly establish the very important role played by the cis-3,4-disubstituted tetrahydrofuran ring in establishing the relative reactivities of the β-hydroxy and five-cyclic diesters (46b and 47b). Even when the cyclization rate of the conjugate base of 56 is considered, the behavior of Brown's model is still sharply different from the behavior of the ribonucleic acids. Our system is by no means the ultimate model of a ribonucleic acid, but the behavior of the conjugate base of 46b is certainly very similar to the observed behavior of the naturally occurring esters.

The unexpectedly high reactivities of the β-hydroxy diesters have been ascribed in great measure to an entropy "boost" structurally built into these esters by virtue of the close proximity of the nucleophilic group to the reaction center. An extension of this reasoning implies that the tetrahydrofuranyl ester (46b), in which both the β-hydroxy and phosphoryl groups are rigidly held in a conformation favorable to the displacement reaction, would possess an entropy "boost" relative not only to the normal diesters but also to other, acyclic β-hydroxy compounds which lack a favorable conformational rigidity. Associated with the β-hydroxy esters may also be an enthalpy "boost" stemming from a non-linear basicity-nucleophilicity relationship among the alkoxide nucleophiles. (See Chapter I.) Brown and Usher (22) have hinted at this proposal but have not discussed their supporting data in this regard. If entropy and enthalpy "boosts" are both responsible for the enhanced internal transesterification rates, these effects should be manifest in

the activation parameters of the appropriate reactions. Therefore, understanding that these observed parameters are the sums of the appropriate parameters for both an equilibrium and a rate step, we have measured the $\Delta H^{\ddagger}$ and $\Delta S^{\ddagger}$ values for the base-catalyzed cyclization of the geometrically restricted 46b (Eq. 40 and Table 9). These values together with the appropriate values for the transesterification of 56 (calculated from Brown and Usher's data at 60 and 80°) and the saponification of Carré's salt (Table 14) are listed in Table 15.

In a comparison of activation entropies, the effects of ionic strength must be considered. The Eyring equation contains the activity coefficient term $\ln \gamma_E \gamma_{OH}/\gamma_{\ddagger}$ which has been conveniently neglected* in the calculations and comparisons of the results given in Table 14. Nevertheless, $\Delta S^{\ddagger}$ values for reactions in solution are dependent upon the choice of standard states, and hence the effect of ionic strength on the activity coefficient term in the Eyring equation cannot be safely neglected. If the $\Delta S^{\ddagger}$ values given in Table 15 had been calculated from rate data collected at constant ionic strength, one would feel reasonably confident in neglecting this term. Unfortunately, Brown's rates have been measured at an ionic strength of 1.0 while our data refer to solutions of ionic strength 0.30. Ionic strength corrections must therefore be considered if the activation entropies are to be based on the same hypothetical standard state of unit concentration (50). The activity coefficients of the appropriate substrates as a function of ionic strength are, of course, unknown but have been approximated using

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* The $\Delta H^{\ddagger}$ values have been assumed to be very insensitive to ionic strength changes in the range 0.2-1.0.

published values as tabulated by Lewis and Randall (81). By substituting ln $\gamma_E\gamma_{OH}/\gamma_{\ddagger}$ values ranging from -0.37 to -0.51 into the Eyring equation, $\Delta S^{\ddagger}$ corrections of about +0.5 to +0.7 e.u. (μ = 0.30) and +1 e.u. (μ = 1.0) are obtained. These corrections have accordingly been applied to the $\Delta S^{\ddagger}$ values listed in Table 15. The relatively small $\Delta S^{\ddagger}$ corrections certainly reinforce our confidence in the $\Delta\Delta S^{\ddagger}$ comparisons obtained from these results.

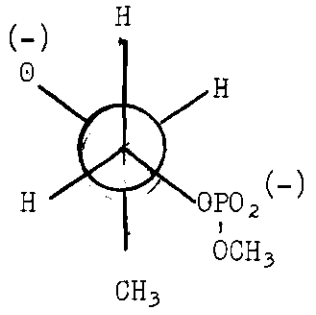
Table 15. The Activation Parameters for the Base-Catalyzed Cyclizations of 46b and 56 and the Saponification of 47b.

Compound	μ	$\Delta H^{\ddagger}$ kcal/mole	$\Delta S^{\ddagger}$ e.u. (uncorrected)	$\Delta S^{\ddagger}$ e.u. (corrected)
46b	0.30	13.7	-27.5	-27
56	1.0	15.5	-32.7	-32
47b	0.30	12.8	-30.3	-30

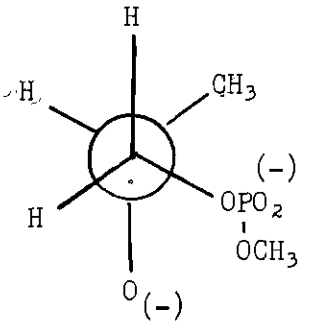
As discussed above, the activation enthalpy for the base-catalyzed P-O fission hydrolysis of dimethyl phosphate is not precisely known but probably lies between 19 and 23 kcal/mole. For comparison purposes, therefore, I have chosen a minimum value of 19 kcal/mole although the true value is probably somewhat larger. The comparison of this number with the $\Delta H^{\ddagger}$ values given in Table 15 for the two transesterification reactions then enables one to conclude definitely that the two β-hydroxy compounds (46b and 56) possess a distinct activation enthalpy

advantage over the simple dimethyl phosphate. Since these enthalpy advantages are alone responsible for rate differences ranging from at least 10^2 to at least 10^4, one must conclude that these two relatively large $\Delta\Delta H^{\neq}$ ($\geq$ 3.5 - 5.3 kcal/mole) values play a major role in the enhancement of the cyclization rates of the β-hydroxy compounds relative to the hydrolysis rate of dimethyl phosphate. Even though terms for both an equilibrium and a rate process are represented by the measured $\Delta H^{\neq}$ values (Eqs. 40 and 43) the large enthalpy advantages constitute good evidence that the alkoxide anions are much better nucleophiles toward phosphorus than is hydroxide ion. (See Chapter I.)

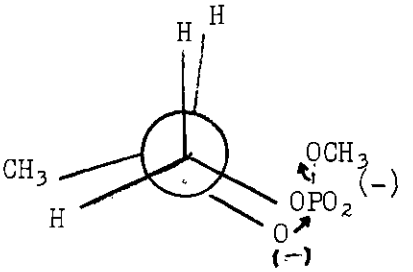
A second, interesting comparison of the data in Table 15 comes from a close inspection of the $\Delta H^{\neq}$ parameters for the transesterifications of 46b and 56. This $\Delta\Delta H^{\neq}$ value of about 1.8 kcal/mole, though fairly small, is probably real and may be explained by a consideration of the relative ground and transition state conformations for the cyclizations of the two β-hydroxy diesters. The most favorable rotational ground states of the alkoxide anions of 56 are represented by 58a and 58b. In order for the cyclization reaction to occur, the ester must rotate into the nearly eclipsed conformation represented approximately by 58c. In as much as there is no X-ray structural information available for a good transition state model (such as a non-aromatic, saturated five-membered cyclic phosphorane), a reasonable quantitative estimate of the energy difference between the ground state conformations (58a and 58b) and the transition state conformation (58c) must remain moot. The ground and transition state structures of the β-hydroxy tetrahydrofuranyl ester (46b) are represented approximately by 59a and 59b, respectively (looking down the C_3-C_4 bond).



58a

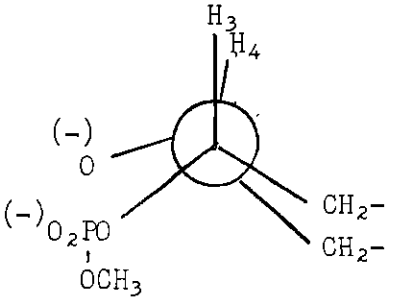


58b

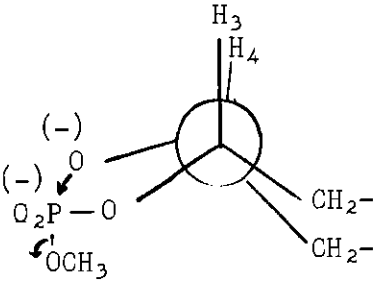


58c

Since the ground state of this ester is held in a favorable, rigid conformation (59a) relative to the ground state conformations (58a or 58b), the energy difference between 59a and 59b would not be expected to be as large as the corresponding differences between the ground states 58a and 58b and the transition state 58c. In partial support of this hypothesis are our n.m.r. results (29) which suggest that a H_3-C_3-C_4-H_4 dihedral angle change of only about 12° takes place on going from 59a to 59b.



59a



59b

A significantly larger angle change would be expected on going from 58b to 58c. It is not at all unreasonable, then, that the change 58a or 58b → 58c would be less favorable energetically by 1-2 kcal/mole than the

change 59a → 59b. This observed $\Delta\Delta H^{\ddagger}$ may also be interpreted as evidence that the ΔH for the equilibrium step in equation 40 is smaller than the analogous equilibrium $\Delta H^{\ddagger}$ in equation 43. In all probability both the equilibria and the conformational effects make contributions to the observed $\Delta\Delta H^{\ddagger}$ value.

It should be recalled that the immediate products of the transesterification reactions, the five-cyclic phosphates, are "strained." Yet, it does not necessarily follow that the transition states themselves leading to the strained five-cyclic esters are strained. Indeed, the relative small $\Delta H^{\ddagger}$ values associated with the cyclizations of 46b and 56 present a very strong case in behalf of the argument that these transition states are essentially strainless, regardless of whether the basal or axial attack transition state model is preferred (Chapter I). If the attack on 47b and 57 by aqueous methoxide and hydroxide follow similar pathways, then the law of microscopic reversibility predicts that the base-catalyzed transesterification transition states are strainless. A thorough analysis of these reactions in acid, where prior phosphoryl migration is expected (19), will probably shed a great deal of light on the nature of these transition states and may demonstrate the validity of this hypothesis.

The activation entropy for the exclusive P-O fission, base-catalyzed hydrolysis of dimethyl phosphate is unknown. Furthermore, any reasonable estimate of this number is rendered impossible by the large uncertainty in determining the appropriate activation enthalpy*. Thus,

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* It must be recalled that the activation enthalpy of 19 kcal/mole chosen above represents a minimum value to be expected for this number.

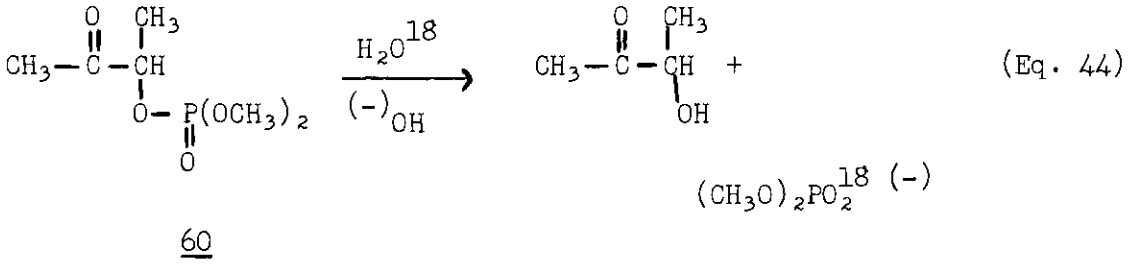
although the $\Delta S^{\ddagger}$ values for the cyclization of the β-hydroxy diesters are probably significantly larger than the $\Delta S^{\ddagger}$ for the P-O cleavage saponification of dimethyl phosphate, there is available no unambiguous experimental evidence to prove this point. It has been predicted, however, that the $\Delta S^{\ddagger}$ for the cyclization of 46b should be larger (i.e., more positive) than the analogous $\Delta S^{\ddagger}$ for the cyclization of 56. This prediction follows from a consideration of the relative ground and transition states for these two transesterification reactions. The ground state of the simpler 56 can, of course, undergo free rotation (58a and 58b); in contrast, the ground state (59a) of the relatively rigid 46b can experience only slight conformational changes resulting from the various puckerings of the tetrahydrofuran ring. Hence, the relatively rigid 46b should lose less entropy in reaching the rigid transition state (59b) than should the freely rotating 56 lose in reaching the rigid transition state (58c). The observable result should then be manifest in a more positive $\Delta S^{\ddagger}$ value for the cyclization of 46b relative to the analogous cyclization of 56. The $\Delta S^{\ddagger}$ values given in Table 15 do indeed demonstrate this expectation. Furthermore, the $\Delta\Delta S^{\ddagger}$ value of 5 e.u. (responsible for a rate factor of about 10 at room temperature) is well beyond the estimated error of $\pm$ 1.5 e.u. associated with each $\Delta S^{\ddagger}$ value reported. But the $\Delta S^{\ddagger}$ parameters, like the corresponding $\Delta H^{\ddagger}$ values, are composite quantities made up of contributing terms for both an equilibrium and a rate step (Eqs. 40 and 43). Tempting as it may be to assume that the observed $\Delta\Delta S^{\ddagger}$ value is representative of only the two rate processes (i.e., $\Delta\Delta S$ equilibrium $\approx$ 0), experimental evidence in support of this position is lacking, and one must therefore be satisfied with the fact that the expected entropy advantage is observed. Actually,

experimental evidence pertaining to the detailed mechanism of the base-catalyzed depolymerizations of the ribonucleic acids is also lacking. The depolymerization reaction, then, may also proceed through intermediate alkoxide ions, in which case the pathways yielding the observed entropy and enthalpy advantages would be directly comparable to the pathway of the nucleic acid breakdown.

Perhaps the most surprising aspect of our comparisons of 46b with 56 lies in the fact that the enthalpy advantage ($\Delta\Delta H^{\ddagger}$) is really larger than the entropy advantage ($T\Delta\Delta S^{\ddagger}$)! In this regard the kinetic behavior of more sophisticated models as well as the nucleic acids themselves, where the rapid cyclization rates have been attributed in large part to the entropy advantage (see reference 34), is of vital interest.

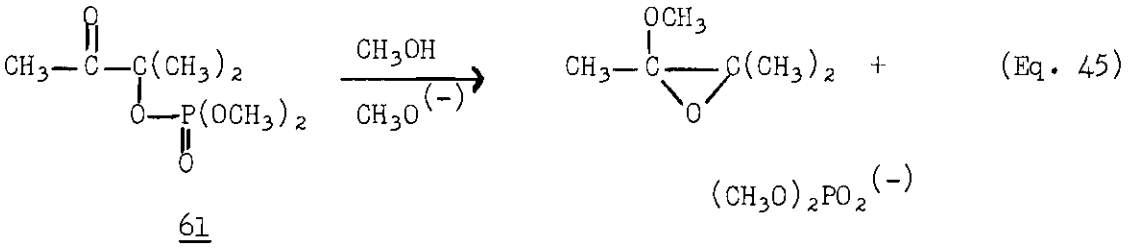
It must be noted that the comparison model (56) has been chosen carefully because it is a secondary alcohol as well as a β-hydroxy methyl ester. As one may have expected, not only the cyclization products (cyclic phosphates or epoxides) but also the cyclization activation parameters are rather sensitive to structural changes in the cyclizing β-hydroxyalkyl group and the alkoxide ion leaving group (22). One may also have expected the β-keto esters to behave much like the β-hydroxy esters. This does not appear to be quite the case, however, at least in the β-keto triester system. Whereas Cox and Farmer (48) have observed that the very rapid, alkaline hydrolysis of dimethyl acetoin phosphate, 60, in O^{18} labeled solvent yields only O^{18} labeled dimethyl (not monomethyl) phosphate (Eq. 44), Cox and I (33) have found that the methoxide ion-catalyzed solvolysis of dimethyl methylacetoin phosphate, 61, in anhydrous methanol apparently yields only the epoxide, 62,

and <u>dimethyl</u> (not trimethyl) phosphate (Eq. 45). Although the β-keto diesters have not been studied, these preliminary observations exemplify



(Eq. 44)

60



(Eq. 45)

61

quite clearly the potential dangers in assuming nearly identical behaviors for two formally similar systems.

Since the intramolecular transesterification reactions of the two β-hydroxy diesters involve both equilibria and rate processes, it has become desirable to examine the kinetic behavior of a system embodying the same (or very similar) relative structural characteristics present in the two β-hydroxy esters but one in which the two equilibria processes have been eliminated. We have therefore studied the kinetics of the two intramolecular S_N2 displacement reactions represented by equations 32 and 33 (Chapter III). The appropriate activation parameters have been calculated from the rate data contained in Tables 11 and 12 and are tabulated in Table 16. In as much as both reactions have been studied at a single ionic strength, the activity coefficient term in

Eyring's equation has been ignored.

Table 16. The Activation Parameters for the Intramolecular S_N2 Displacement Reactions of the Bromoalkyl Phosphates (48 and 49).

Compound	μ	$\Delta H^{\neq}$ (1) (kcal/mole)	$\Delta S^{\neq}$ (2) (e.u.)
2-Bromoethyl Phosphate (48)	0.20	24.6	+2.1
cis-3-Bromotetrahydrofuranyl-4-phosphate	0.20	28.5	+4.9

(1) ± 0.7 kcal/mole; (2) ± 1.0 e.u.

The argument employed to predict the activation entropy differences for the transesterifications of 46b and 56 applies qualitatively to these intramolecular S_N2 displacement reactions. Since the ground state of 48 is subject to free rotation while the ground state of 49 is relatively rigid, the activation entropy for cyclization would be expected to be more negative for the non-rigid 48 than for the rigid 49. The $\Delta S^{\neq}$ values presented in Table 16 verify this prediction. In examining the magnitude of the observed $\Delta\Delta S^{\neq}$ value, one must realize that the secondary bromide, 2-bromopropyl phosphate should be a better comparison model than the 2-bromoethyl phosphate. A consideration of the S_N2 transition state model of Dostrovsky, Hughes, and Ingold (42) taken with the findings of Hammett and coworkers (37,44) on the reactivities of the ethyl and n-propyl halides, indicates that the decrease in the activation entropy

accompanying an increase in the size of the β substituent may be due to the increasing interference to rotation of the substituent around the C_α-C_β bond.* The application of this argument to our system implies that the cyclization of 2-bromopropyl phosphate would show a more negative $\Delta S^{\neq}$ than has been observed in our study of 2-bromoethyl phosphate. The $\Delta\Delta S^{\neq}$ value from Table 16, reinforced by this argument, then establishes rather firmly the hypothesis of an activation entropy advantage associated directly with the rigidity of the trans-3,4-disubstituted tetrahydrofuran ring. The logical, qualitative extension of this finding almost definitely implicates the cis-3,4-disubstituted tetrahydrofuran ring with the observed entropy advantage associated with the intramolecular transesterification of 46b! One must now be very careful to realize that this conclusion is of a qualitative nature only! Since the transition states of the transesterification reactions (Eqs. 40 and 43) are quite different from the transition states of these internal S_N2 displacement processes, any attempt at a direct, quantitative comparison of the data in Tables 15 and 16 would be ridiculous.

It is interesting that the S_N2 cyclization reactions of the phosphates are rather similar to the analogous lactonization reactions in the carboxylic acid series (62,79). The observed positive $\Delta S^{\neq}$ values demonstrate that the transition states are less polar than the reactants for the displacement reactions in both series of compounds. That the more positive $\Delta S^{\neq}$ values are associated with the lactonization processes is really not surprising when one considers the relative ground and

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* This topic has been discussed by Hine (64).

transition state solvations of the mono- vs. the divalent anion nucleophiles. It must also be understood that the S_N2 transition states leading to the five-cyclic phosphates are by no means strainless. On the other hand, the $\Delta\Delta H^{\neq}$ value obtained from Table 16 is certainly no indication of the relative strains in ethylene phosphate (12) and Carré's salt (47b). The inductive effect of the ether oxygen in the tetrahydrofuran ring would be expected to raise the S_N2 cyclization $\Delta H^{\neq}$ parameter of 49 relative to 48. An additional, and probably more important factor, is apparent from an examination of molecular models of 49. This examination suggests that the rigidity of 49 tends to restrain this ester from assuming the optimum (i.e., linear) configuration for an S_N2 transition state, thereby further increasing the energy requirements for reaching such a transition state. In this respect it seems somewhat ironic that the conformational rigidity mainly responsible for the entropy advantage should be partially responsible for the enthalpy disadvantage.

CHAPTER V

CONCLUSIONS AND RECOMMENDATIONS

Throughout Chapters I and IV I have tried to offer reasonable interpretations of the available experimental evidence and to suggest other experiments which I consider to be potentially worthwhile. Many assumptions have been made, and much experimental work remains to be done. I feel, therefore, that a brief recapitulation of the most important conclusions derived from our own work is in order. On the basis of these conclusions I am suggesting those experiments which I consider to be the most significant outgrowths of this research.

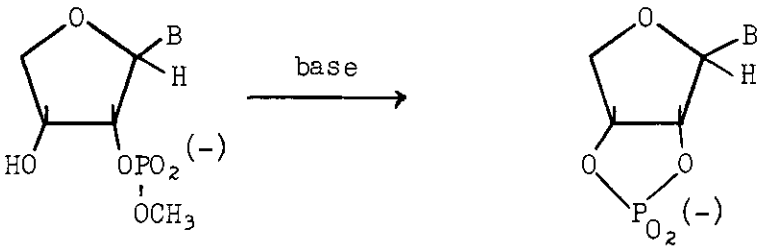
1. Our activation parameters for the base-catalyzed hydrolysis of ethylene phosphate, taken with the published rate data for the saponification of the acyclic dimethyl phosphate, present a strong argument that the five-membered phosphate diester ring contains more than the 5.5 kcal/mole of ring strain found in the five-membered triester. These data further suggest that the phosphorus atom in the five-membered ring ester bears an enhanced positive charge relative to the phosphorus atom in an acyclic ester. If these tenets are to be confirmed, the base-catalyzed hydrolysis activation parameters for the five-cyclic methyl ethylene phosphate must be measured and compared with the analogous data for trimethyl phosphate.

2. The rate and activation data obtained for the base-catalyzed hydrolysis of Carré's salt prove that the presence of the cis-3,4-tetrahydrofuran ring has almost no observable effect on the hydrolytic

reactivity of the five-membered phosphate grouping toward aqueous base. The acid-catalyzed hydrolysis of this ester must, of course, be measured if this conclusion is to be generalized. In addition, better, more sophisticated cyclic nucleotide models (such as 64) must be studied if the effects of the various groups attached to the ribose ring are to be evaluated (Eq. 47). As the structures of the models approach more closely the structures of the cyclic nucleotides, the possibility of enzymatic catalysis becomes an extremely important consideration. This catalysis, if detected, must be studied carefully.

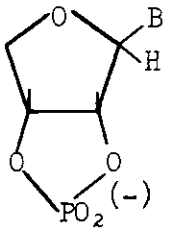
3. The comparison of the rate data on the base-catalyzed transesterifications of 56 (22) and 46b points out the very large effect exerted by the tetrahydrofuran ring on the ease of cyclization of 46b. This effect is manifest in both entropy and and enthalpy terms. Moreover, these and other data strongly suggest that the enhanced reaction rates of the β-hydroxy diesters in general are due to both entropy and enthalpy advantages associated directly with the β-hydroxyl groups. A study of a suitable β-hydroxy triester should be undertaken with regard to this last conclusion. Again, better ribonucleic acid models (as 63) must be studied (Eq. 46), and a search for enzymatic catalysis must be undertaken.

4. The activation parameters for the base-catalyzed transesterification of 46b provide good evidence that the transesterification transition states are "strainless." The corresponding acid-catalyzed cyclization studies may well shed considerable light upon this hypothesis as well as prove to be mechanistically important.



(Eq. 46)

63 64



(Eq. 47)

64

5. Our failure to observe general base catalysis in the transesterification of 46b implicates a two-step sequence for this reaction. Our result, however, does not eliminate this possibility, and indeed a general base-catalysis mechanism may be found in the cyclization of the related dimethyl triester, 65, substituted phenyl diester, 55, or the models 63 and 64. The search for general base and general acid catalysis is really a "must" if enzymatic processes are to be considered!

65 55

6. The kinetic data on the internal S_N2 cyclization reactions of 2-bromoethyl and trans-3-bromotetrahydrofuranyl-4-phosphates show that an activation entropy advantage is associated with the conformational rigidity of the tetrahydrofuran ring. The application of this conclusion to the internal transesterification of 46b all but confirms that the entropy advantage observed in this transesterification reaction is associated with the conformational rigidity of this ester. Hence, the entropy advantage postulated for the depolymerization of the ribonucleic acids appears quite real although its true magnitude remains to be determined.

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VITA

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